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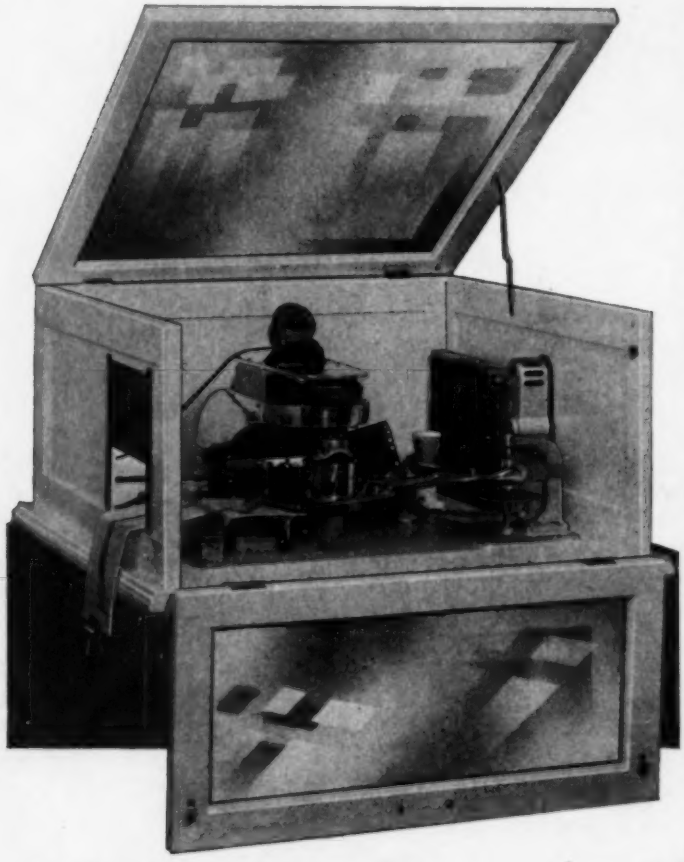

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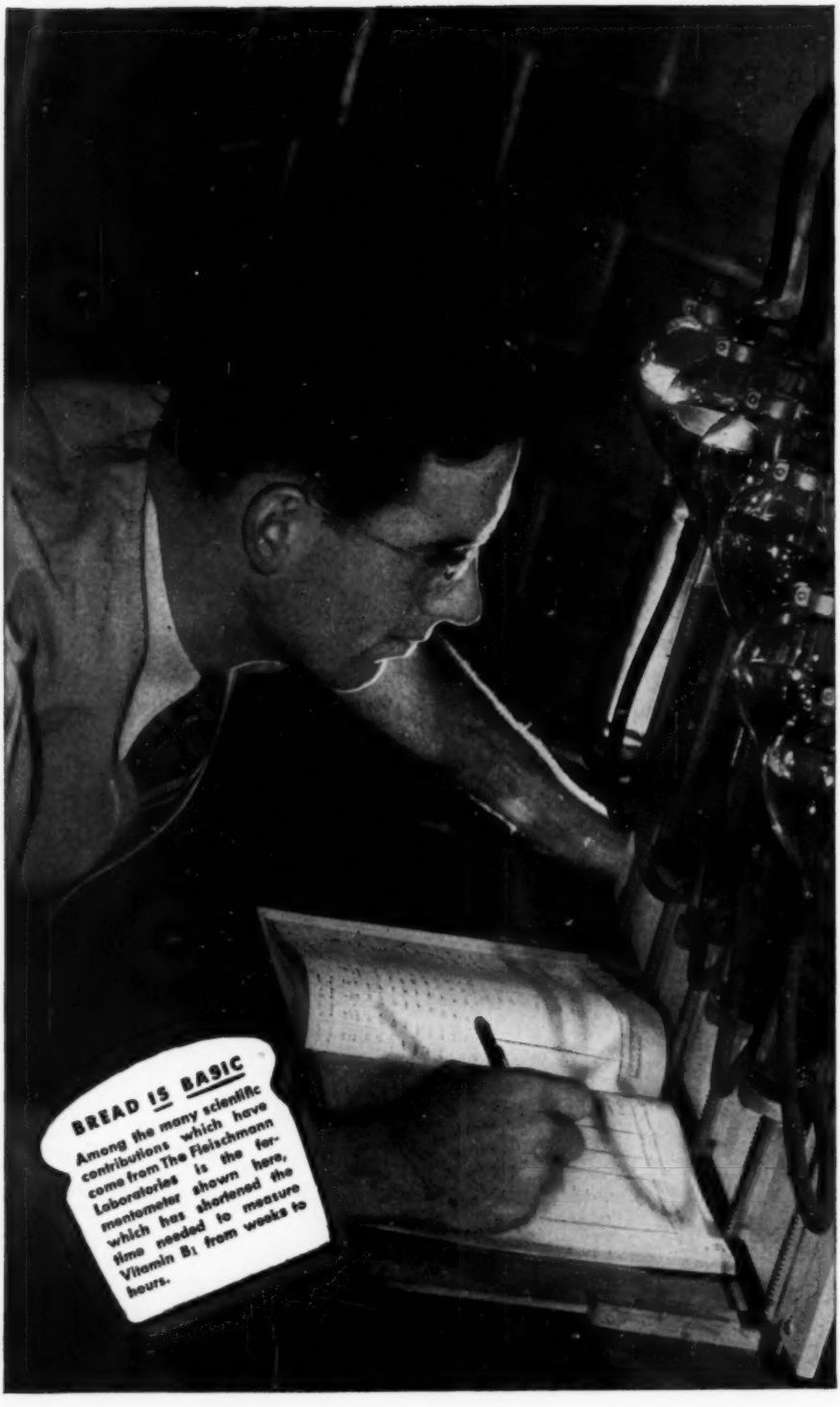
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CEREAL CHEMISTRY

VOL. XX JULY, 1943 No. 4

THE DIFFERENTIAL STABILITY OF THE MALT AMYLASES—SEPARATION OF THE ALPHA AND BETA COMPONENTS [1]

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(Read in part at the Annual Meeting, May 1942)

The conclusion of Märker (1879) that malt diastase is composed of two active starch-degrading components led to the development of many methods for the separation of these two enzymes. These methods are based on variations in such properties as adsorption, surface denaturation, solubility in alcohol or salt solutions, and response to combinations of temperature and hydrogen-ion concentration. The experiments reported in this communication deal with the response of the malt amylases to various combinations of temperature and hydrogen-ion concentration and the manner in which these enzymes are precipitated by alcohol and by solutions of ammonium sulfate.

Although alcohol was used as a means for concentrating "malt diastase" by some of the earliest workers (Payen and Persoz, 1833) and the differential solubility of the two malt amylases in alcohol was recognized by Wijsman (1890), it was not until the work of van Klinkenberg (1931, 1932) that this agent was suggested as a practical means of separation. He found that the two components tended to precipitate at different concentrations of alcohol and used 60% alcohol for the precipitation of alpha-amylase, followed by an increase to 80% to precipitate beta-amylase. Caldwell and Doebbeling (1935) confirmed this finding and in addition indicated that barley malt alpha-amylase could be differentially precipitated by concentrations of ammonium sulfate below the 20% to 35% region in which most of the beta component became insoluble. Here again the technique employed

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a classical protein precipitant, in this case one popularized by Osborne (1895).

Recently the most generally used means for separating the two amylases of malt has been the technique of Ohlsson (1926). O'Sullivan (1876) early observed that heating a malt extract to 75°C resulted in a product of high dextrinizing and low saccharifying powers when compared to the original unheated extract. This was confirmed by Märker (1879) and used by him to postulate the existence of two amylase components in malt. Brown and Heron (1879) presented further data on thermal inactivation and also observed that slight alkalinity had an effect similar to heat in differentially inactivating the saccharifying power of malt extract. The work of Bourquelot (1887) threw additional light on the manner in which heat inactivates the malt amylases, but it was not until 1890 that definite proof of the existence of two amylase components was furnished. In this year Wijsman (1890) published evidence that the saccharifying component differs from the dextrinizing amylase in being more soluble in alcohol, more sensitive to heat and to alkali, and less sensitive to acid. He likewise demonstrated that the saccharifying enzyme diffuses more rapidly through a gelatin-starch medium.

Based on the above findings Ohlsson (1922, 1926, 1930) devised a technique whereby beta-amylase could be inactivated by heating a malt extract at a pH close to neutral for 15 minutes at 70°C. Conversely a treatment at ice-water temperature and pH 3.3 for 15 minutes resulted in the inactivation of most of the alpha-amylase with but little effect on the beta component. This technique has been used extensively by many workers but apparently not always with the realization that neither treatment results in complete recovery of the desired component nor invariably in complete inactivation of the undesired amylase.

Other amylase stability factors considered in the present treatment are the influence of extract dilution and the role played by the calcium ion. Sherman and Schlesinger (1915) and Venkata Giri and Subrahmanyan (1932) have noted that amylase loses its activity a great deal more rapidly in dilute than in concentrated extracts. The latter authors also found that the starch-liquefying activity of malt extracts deteriorates more rapidly than does the saccharifying activity. With regard to the influence of calcium ions on the stability of the amylases Nakamura (1931) in the last of a series of papers on amylase-protecting substances concluded that calcium salts have a pronounced effect in protecting amylases from inactivation by heat. Wallerstein (1909) early recognized the stabilizing action of calcium and patented its use for such purposes. Hollenbeck and Blish (1941) confirmed these

earlier findings and in addition their data showed that the protective action of the calcium ion is of large magnitude.

The conclusions derived from these stability studies may be accepted only with reservations by reason of the inadequacy of some of the prevalent methods. It was deemed advisable to re-examine some of the conceptions, making use of the differential methods recently devised by Sandstedt, Kneen, and Blish (1939) and Kneen and Sandstedt (1941) for the determination of alpha- and beta-amylase activities. Too, most of the published data deal exclusively with barley malt. The current industrial importance of wheat malt necessitates similar investigations of this cereal. In addition it is desirable that methods be devised for the preparation of either amylase component uncontaminated by the other. The present study is concerned with the differential stability of the amylases of barley and wheat malts and with the feasibility of isolating "pure" preparations.

Methods

Determination of amylase activities: The methods of Sandstedt, Kneen, and Blish (1939) and of Kneen and Sandstedt (1941) were used for the determination of the activities of the individual amylase components. Where recorded, the amylase "units" are those proposed by these authors. The exception to the above was in the determination of relatively micro-quantities of alpha-amylase. In this instance it was necessary to use a micro method. An amount of extract equivalent to a fairly large weight of material (0.1 to 1.0 g, depending on the activity) is allowed to act on 20 ml of beta-amylase-treated 2% "Lintner starch" for some 16 to 18 hours at 30°C. At the end of this period constant amounts of malt extract are added to the unknown and to a blank beta-amylase-treated starch and detrinization times measured. Any shortening of the blank dextrinization time is proportional to the degree of dextrinization which took place in the prolonged action of the unknown sample. This in turn is proportional to the amount of alpha-amylase in the unknown sample. Units are calculated as

$$\frac{24}{\text{weight of material used} \times \text{total elapsed time}} \times \frac{\text{blank dextrinization time}}{\text{blank} - \text{unknown}}$$

For example, suppose that the equivalent of 0.1 g of an ungerminated cereal worked on the substrate for 18 hours. At the end of this time malt was added to this and to an additional portion of the substrate in equivalent amounts. The blank dextrinization time was 20 minutes

but the dextrinization time on the partially converted sample was 10 minutes. The ungerminated cereal would have an alpha-amylase activity of

$$\frac{24}{0.1 \times 1080 \times \frac{20}{20 - 10}} \text{ or } 0.11 \text{ unit.}$$

Alcohol and salt precipitation: All these precipitations were carried out at ice-water temperatures. The resulting precipitates were centrifuged out at high speed in an angle centrifuge and then dissolved in water. The water solutions of the alcohol precipitates were used as prepared; those of the ammonium sulfate precipitates were dialyzed against running tap water for 48 hours before use.

Thermal and acid treatments: In various instances temperatures ranging from that of ice water to 70°C and hydrogen-ion concentrations from pH 2.6 to 7.0 were used. In all cases precautions were taken to insure constancy of pH, uniformity of treatment, and minimal loss from evaporation. The pH values were determined or adjusted as desired by the use of glass-electrode equipment. Where temperatures higher than 30°C were used or pH values below 4.5, the solutions were rapidly brought to these relatively optimum conditions after the desired treatment and before activity determinations were made. Studies at low pH values (in the neighborhood of 3.0) were all carried out in a uniform manner: the pH of the extract was lowered to that desired by the use of 0.1*N* hydrochloric acid, held at that pH for the designated period of time, then rapidly raised to between 4.5 and 5.0 by the use of either 0.1*N* sodium hydroxide or 8% sodium acetate.

In those experiments involving the influence of calcium ion at various hydrogen-ion concentrations and temperatures, the addition of calcium ion was in the form of either the chloride or the acetate salt. In the range of concentrations used there was no detectable difference in effect between these two calcium salts.

Extraction of malts: Unless otherwise indicated the extractions were for 1 hour at 30°C using distilled water as the extractant. For the barley-malt extracts the freshly and finely ground meal from dry malt grain was used. The wheat-malt extracts were obtained from a commercial wheat-malt flour. Extract concentrations are referred to as 1-to-5, 1-to-10, etc., thus indicating the ratio of meal to extractant: 1 g meal to 5 ml extractant, 1 g to 10 ml, etc.

Results

Ammonium sulfate precipitation: Table I shows the fractions of the total ammonium-sulfate-precipitable amylases deposited by the indi-

cated increment of this salt. It should be emphasized that an indicated amylase percentage is that precipitated within a certain range of salt concentration. For example, in the case of the barley-malt alpha-amylase, 2.0% of the enzyme was precipitated by 10% concentration of ammonium sulfate, an additional 7.8% by increasing the salt content to 15%, an additional 37.3% in the range between 15% and 20% salt, and so on until all the salt-precipitable alpha-amylase was removed from solution at 40% salt concentration. In this instance increasing the salt content to 45% concentration resulted in no *additional* activity in the precipitate.

TABLE I

THE PRECIPITATION OF MALT AMYLASES BY AMMONIUM SULFATE
(Fractions of amylases precipitated)

Ammonium sulfate increment	Barley malt		Wheat malt	
	α-amylase	β-amylase	α-amylase	β-amylase
%	%	%	%	%
0–10	2.0	0.4	4.4	2.7
10–15	7.8	1.5	2.2	3.2
15–20	**37.3**	1.1	8.9	10.6
20–25	**35.3**	12.5	13.3	12.2
25–30	11.8	**39.7**	22.2	22.8
30–35	3.9	**38.6**	**37.8**	**37.7**
35–40	2.0	6.2	8.9	10.8
40–45	0.0	0.0	2.2	0.0
45–50	0.0	0.0	0.0	0.0

The data of Table I indicate that there are differences in the manner in which the amylases of barley- and wheat-malt extracts respond to ammonium sulfate precipitation. The barley-malt amylases precipitated in a manner similar to that reported by Caldwell and Doebbeling (1935). Most of the alpha-amylase was precipitated between the 15% and 25% levels of ammonium sulfate. It was necessary to increase the salt concentration to 35% to remove most of the beta-amylase, the greater part of this component being precipitated in the region between 25% and 35% ammonium sulfate concentration. No such differential effect as this was found for the wheat malt. In two separate trials, of which only one is reported, the alpha- and beta-amylases of wheat malt responded almost identically to ammonium sulfate precipitation. In both cases the most active precipitates were those resulting when the salt concentration was increased from 30% to 35%.

It is apparent that ammonium sulfate cannot be used as the sole agent for precipitating one of the amylase components to the exclusion of the other. With barley-malt extracts precipitation at the 20% level gave alpha-amylase slightly contaminated with beta. By removing the precipitate resulting from 30% salt concentration, and then increas-

ing the salt content to 35% or 40%, it should be possible to get a precipitate relatively high in beta-amylase activity and low in, but not free from, alpha-amylase activity; about 6% of the alpha-amylase remained and over 50% of the beta-amylase was lost. With wheat malt even this degree of separation of the amylases could not be anticipated by fractionation with ammonium sulfate.

Ethyl alcohol precipitation: The percentages of the total alcohol-precipitable amylases deposited by the indicated increments of ethyl alcohol are shown in Table II. Alcohol concentrations are recorded as percent by volume and, as with the salt-precipitation studies, the designated precipitation of amylase is that occurring within the indicated range in alcohol concentration. With the wheat-malt extract an alcohol concentration of 50% by volume was the lowest concentration at which perceptible precipitation occurred, whereas noticeable precipitation was present in the barley-malt extract at 32% alcohol content. Here, as with the ammonium sulfate precipitation study, there was a definite tendency for the amylases of barley malt to precipitate at lower concentrations of the agent than for those of the wheat-malt extract.

TABLE II

THE PRECIPITATION OF MALT AMYLASES BY ETHYL ALCOHOL
(Fractions of amylases precipitated)

Barley malt			Wheat malt		
Alcohol increment	α-amylase	β-amylase	Alcohol increment	α-amylase	β-amylase
%	%	%	%	%	%
0–32	14.1	5.0	0–50	11.3	5.6
32–38	14.1	0.9	50–56	**61.7**	10.3
38–44	17.6	2.9	56–62	27.1	**41.6**
44–50	**29.4**	11.5	62–68	0.0	**42.5**
50–56	12.9	16.0	68–74	0.0	0.0
56–62	5.9	**53.7**	74–80	0.0	0.0
62–68	5.9	10.1			
68–74	0.0	0.0			
74–80	0.0	0.0			

From the data of Table II it is apparent that alpha-amylase precipitates at lower concentrations of alcohol than does beta-amylase. This was true for both wheat and barley malts but more strikingly so with respect to barley. Some degree of separation of the two amylases by use of alcohol precipitation appears possible but not in a quantitative sense. At no concentration was alpha-amylase precipitated free from beta-amylase. However, with the wheat malt, all the alpha-amylase appeared to be precipitated by 62% alcohol, leaving some 40%

of the beta-amylase still in solution. Even here the apparent absence of precipitable alpha-amylase does not preclude the possibility that this enzyme was present in micro quantities not detected by the macro method used.

Thermal inactivation: No study of the manner in which the malt amylases are inactivated by heat can ignore consideration of the hydrogen-ion concentration of the solution. However, in certain ranges the influence of temperature is predominant. Too, as has already been noted, the concentration of calcium ion markedly influences the thermostability of the amylases and this factor therefore must be considered.

Laboratory observations have shown that dialysis against tap water previous to the differential destruction of beta-amylase by heating a malt extract results in a good yield of alpha-amylase coincident with a rapid inactivation of beta-amylase. Table III shows a comparison of the amylase stabilities in distilled-water extracts of barley and wheat malts before and after a two-day dialysis against running tap water. One to 5 extracts of the malts (1 g malt to 5 ml water) were treated at two temperatures, 60° and 70°C. Aliquots for the determination of alpha- and beta-amylase activity were taken at the time the solution reached the desired temperature and following the indicated intervals at that temperature. It should be noted that there is an unavoidable loss in activity during the time required to raise the extract temperature from 30°C to that used for the treatment. Also two additional variables were operative in influencing the stability of the malt at any one temperature: hydrogen-ion concentration and calcium-ion concentration. The pH values of the original undialyzed extracts were 5.0 for the wheat malt and 5.5 for the barley malt. In contrast to these after dialysis against tap water the pH value was 7.0 for both extracts. Dialysis likewise resulted in a notable change in the calcium-ion content of the extracts: in the case of the barley malt extract an increase from 0.06 mg to 0.11 mg of calcium per milliliter.

The data of Table III show that with either barley- or wheat-malt extracts, tap-water dialysis brought about an increase in the thermostability of alpha-amylase and a corresponding decrease in the stability of beta-amylase. This was true at either 60° or 70°C. With the undialyzed wheat-malt extract more than two-thirds of the alpha-amylase activity was lost by a 15-minute treatment at 70°C, whereas the same treatment of the dialyzed extract resulted in a loss of less than 5%. With either malt a 60-minute treatment at 60°C was not sufficient to inactivate all the beta-amylase in the undialyzed extracts, whereas 5 minutes or less at this temperature sufficed for complete inactivation with the dialyzed extract. It is apparent from Table III

TABLE III

THE INFLUENCE OF TAP-WATER DIALYSIS ON THE RELATIVE THERMO-STABILITIES OF THE MALT AMYLASES

(Fractions of original activity remaining)

Treatment	Alpha-amylase				Beta-amylase			
	No dialysis		Tap-water dialysis		No dialysis		Tap-water dialysis	
	60°C	70°C	60°C	70°C	60°C	70°C	60°C	70°C
WHEAT MALT								
No heat	100	100	100	100	100	100	100	100
At temp:								
0 min	94	74	97	100	94	15	0.0	0.0
5 min	88	57	97	100	88	0.9	0.0	0.0
10 min	86	43	96	97	60	0.5	—	—
15 min	81	31	96	96	52	0.0	—	—
60 min	59	3.3	94	67	31	0.0	—	—
BARLEY MALT								
No heat	100	100	100	100	100	100	100	100
At temp:								
0 min	97	97	98	96	96	11	17	0.3
5 min	97	95	98	96	76	3.2	0.0	0.0
10 min	97	91	98	96	64	1.3	0.0	0.0
15 min	97	89	98	96	58	0.0	—	—
60 min	97	56	98	84	25	0.0	—	—

that the accepted procedure of heating for 15 minutes at 70°C is adequate to effect removal of beta-amylase activity in the 1-g to 5-ml extracts of barley or wheat malts, both in the original undialyzed extracts and in those dialyzed against tap water. Good recovery of alpha-amylase was attained with barley malt either dialyzed or undialyzed. However, the low pH and low calcium content of the undialyzed wheat-malt extract resulted in a pronounced loss of alpha-amylase. The data indicate that it should be possible to adjust the pH and calcium ion content of an extract so that rapid inactivation of beta-amylase would coincide with maximum recovery of alpha-amylase.

The problem of amylase stability at 70°C is largely that of the effect of this temperature on alpha-amylase. At pH values of 5, 6, or 7, either with or without added calcium, the beta-amylase of either wheat or barley malt extracts was always found to be completely inactivated in 15 minutes and often by the time the 70°C temperature was attained. Figure 1 shows the reaction of malt alpha-amylase to 70°C treatment under various conditions. One to 5 extracts of wheat and barley malts were used at pH values of 5.0 and 6.0. In addition, treat-

ments at pH 6.0 with the addition of 0.4 mg calcium per ml were carried out. Aliquots were withdrawn for activity determinations when the temperature reached 70°C and again at the end of 5, 10, 15, 20, 30, 40, 60, 90, and 120 minute intervals at 70°C. In all instances beta-amylase activity was reduced to a very low level by the time the 70°C temperature was attained.

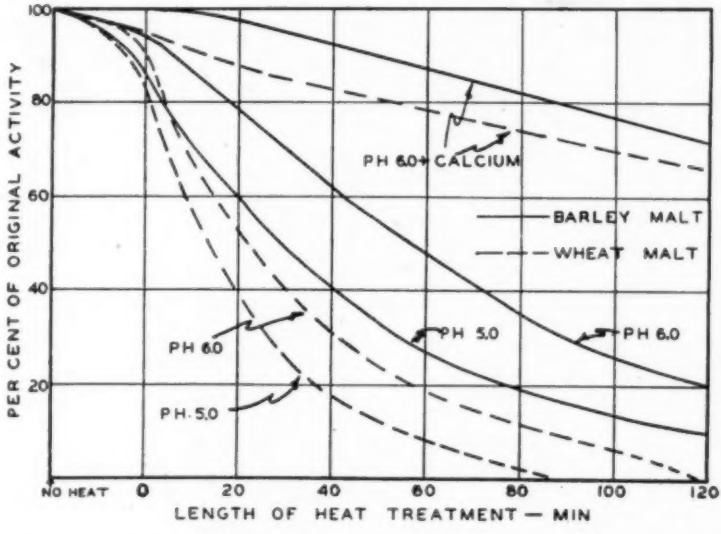

Fig. 1. The influence of pH and of calcium ion on the stability of malt alpha-amylase at 70°C.

The curves of Figure 1 illustrate the dependence of alpha-amylase stability on pH and on the concentration of calcium in solution. Activity was lost more rapidly at pH 5.0 than at 6.0. The addition of calcium ion had a pronounced stabilizing action. With wheat-malt extract at pH 6.0 and no added calcium, all the alpha-amylase activity was destroyed by a 2-hour treatment at 70°C; at the same pH but with the addition of calcium some 67% of the activity remained at the end of the 2 hours of heating.

The above described thermal-inactivation studies were conducted under such conditions (high temperatures and pH values of 5.0 or above) that the major influence was on the beta component of malt amylase. At somewhat lower temperatures and pH values it proved possible to evaluate the sensitivity of the two amylases to conditions unfavorable to stability toward both temperature and hydrogen-ion concentration. Data for wheat malt are shown in Figures 2 and 3. The extracts were in the ratio of 1 part of meal to 10 parts of distilled water and no dialysis treatments or calcium additions were made pre-

vious to use. Samples were withdrawn for activity determinations when the extracts reached a temperature 5° below that desired, again when the experimental temperature was attained, and then following intervals of 10, 20, 30, and 60 minutes of treatment. Processes involving increasing the hydrogen-ion concentration or raising the temperature to the desired level were conducted as rapidly and uniformly as possible.

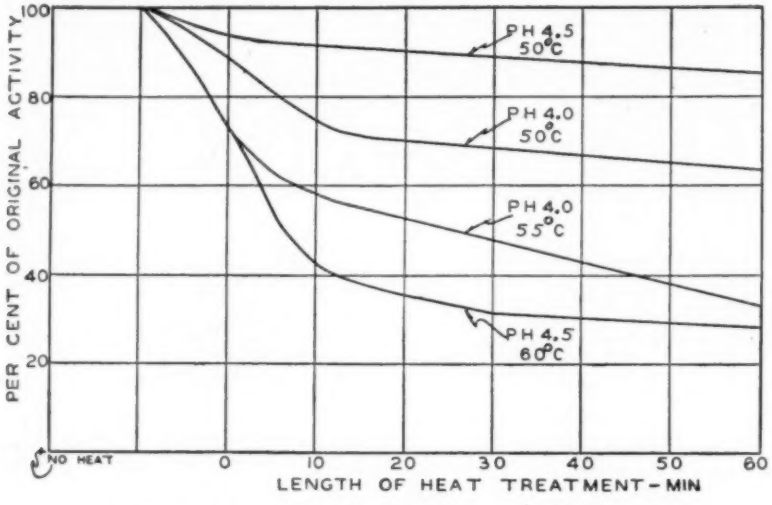

Fig. 2. The influence of combinations of temperature and pH on the stability of wheat-malt beta-amylase.

The curves of Figure 2 show the response of the beta-amylase of wheat malt to the temperature and pH conditions. In no instance was any loss of beta-amylase detected by the time the temperature reached the level 5° below that to be used. Following this point, however, there were measurable losses of beta-amylase in all instances. At the end of 30 minutes' treatment 89% of the activity remained at 50°C and pH 4.5, 69% at 50° and pH 4.0. 50% at 55° and pH 4.0, and 32% at 60° and pH 4.5. It is apparent that temperature was the predominant factor influencing beta-amylase stability. For instance, there was a more pronounced loss of this enzyme at 60°C and pH 4.5 than at 55° and the lower pH of 4.0. However, the pH of the solution did have a pronounced effect; at 50°C the loss in activity was considerably greater at pH 4.0 than at pH 4.5. It may be concluded, then, that under the experimental conditions, stability was dependent upon both temperature and hydrogen-ion concentration with the temperature effect being predominant.

Figure 3 shows the response of wheat-malt alpha-amylase to the same conditions as those reflected by the beta-amylase curves of Figure 2. It may be noted that with alpha-amylase, as with beta, both hydrogen-ion concentration and temperature influenced the stability. However, with this amylase the influence of hydrogen-ion concentration was predominant. At pH 4.0 nearly 60% of the alpha-

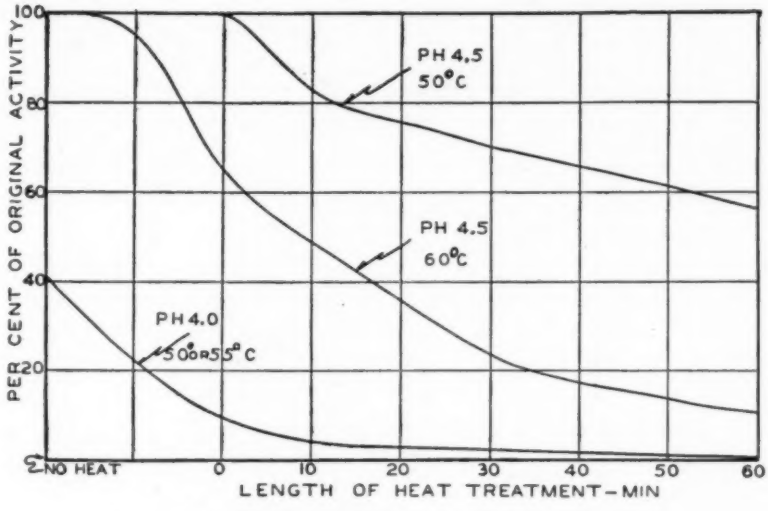

Fig. 3. The influence of combinations of temperature and pH on the stability of wheat malt alpha-amylase.

amylase was inactivated before the procedure of raising the temperature was begun. Further loss of activity was rapid and equal in degree, whether the temperature was raised to 50 or 55°C. Inactivation was much less rapid at pH 4.5 and was inappreciable until a temperature of 50° was attained. At the end of 30 minutes of treatment 71% of the alpha-amylase activity remained at pH 4.5 and 50°C, 22% at pH 4.5 and 60°, and 3% at pH 4.0 and either 50 or 55°C.

Relative stabilities of wheat- and barley-malt amylases: The data of Table III and Figure 1 indicate that barley-malt alpha-amylase is more stable than that from wheat. The data dealing with beta-amylase activity (Table III) are not conclusive, due to experimental conditions being either too severe on beta-amylase or not equivalent for the two malts. Accordingly, in addition to the experiments with wheat-malt extracts, 1-to-10 barley-malt extracts were treated at pH 4.0 and 50°C and at pH 4.5 and 60°C. The results are presented in Table IV along with those found for wheat malt under the same conditions.

TABLE IV

THE RELATIVE STABILITY OF THE AMYLASES OF WHEAT AND BARLEY MALTS
(Fractions of original activity remaining)

Treatment	pH 4.0, 50°C				pH 4.5, 60°C			
	Alpha-amylase		Beta-amylase		Alpha-amylase		Beta-amylase	
	Wheat	Barley	Wheat	Barley	Wheat	Barley	Wheat	Barley
No heat	41	66	100	100	100	100	100	100
At temp:								
0 min	9.2	34	89	96	63	88	74	77
10 min	4.6	8.1	74	68	50	79	42	32
20 min	3.6	5.0	70	53	38	63	36	13
30 min	3.1	4.3	69	42	22	59	32	3.8
60 min	1.9	3.2	63	25	11	41	28	0.0

It is apparent from Table IV that the alpha-amylase of wheat malt was more sensitive to either set of experimental conditions than was barley-malt alpha-amylase. This was particularly evident at pH 4.5 and 60°C. After one hour's treatment under these conditions some 41% of the barley alpha-amylase remained active and only 11% of that in the wheat malt extract. These results assume added significance when those for beta-amylase are considered. This enzyme might be expected to react in a manner similar to that of alpha-amylase. Instead, the data for beta-amylase activity indicate just the reverse, beta-amylase from wheat being more resistant than that from barley. A one-hour treatment at pH 4.5 and 60°C resulted in complete inactivation of barley-malt beta-amylase as compared to a 72% loss of activity with wheat malt.

The data confirm those found by precipitation techniques in indicating real differences in properties between the amylases from barley and wheat: barley alpha-amylase has greater thermostability than wheat alpha-amylase, wheat beta-amylase greater thermostability than barley beta-amylase. The differences in stability may be related to substances, other than calcium, accompanying the enzymes in the extracts; the calcium ion contents in the two extracts were essentially identical. It should be noted here that this particular barley-malt extract had about twice as much alpha-amylase and two and one-half times as much beta-amylase actvitiy as the extract from the wheat malt. This in no way invalidates the conclusions but emphasizes them in the case of beta-amylase. As regards the relative stability of the alpha-amylases, the difference in concentration could be postulated as leading to the results were it not for the data of Figure 1; in those experiments (Fig. 1) two malts of essentially equal alpha-amylase activity were used.

The influence of dilution on amylase stability: Frequently it has been noted that dilute malt extracts tend to lose amylase activity more rapidly than those of greater concentration. For example, when a 1-to-10 and a 1-to-20 extract of barley malt were subjected to pH 4.5 and 60°C for 10 minutes, the 1-to-10 extract retained 79% of the alpha-amylase activity and 32% of the beta-amylase, whereas the 1-to-20 extract had remaining only 32% of the alpha-amylase and less than 1% of the beta-amylase. This difference in stability appears to be a function of extract dilution rather than enzyme concentration. Extracts of two barley malts in the same ratio of malt to water lost their amylase activities at approximately the same rate. This is illustrated in Table V. The data of this table were obtained by comparing extracts of two barley malts at pH 5.0 and 70°C. Both malts were extracted in the same ratio of meal to water, *i.e.*, 1 to 5. Malt A had amylase concentrations of 47.0 units of alpha-amylase and 5.6 units of beta-amylase. Malt B was higher in alpha-amylase (66.7 units) and much higher in beta-amylase (15.6 units).

TABLE V

RELATIVE STABILITY OF THE AMYLASES OF TWO BARLEY MALT EXTRACTS HEATED AT 70°C AND pH 5.0

(Fractions of original activity remaining)

Treatment	Malt A (orig. activ. 47.0 α, 5.6 β)		Malt B (orig. activ. 66.7 α, 15.6 β)	
	Alpha-amylase	Beta-amylase	Alpha-amylase	Beta-amylase
	%	%	%	%
No heat	100	100	100	100
At 60°C	100	79	98	78
At 70°C	97	0	88	0
At 70°C:				
10 min	78	0	70	0
20 min	64	—	61	—
30 min	56	—	49	—
60 min	32	—	27	—

The data of Table V indicate that, notwithstanding the differences in amylase concentrations, both alpha- and beta-amylase showed diminution of activity at approximately the same rate. In fact the amylases of malt B (the malt initially having higher concentrations of both) were inactivated at a slightly greater rate during the first stages of heating. These data suggest that the influence of extract dilution must be due in large part to the effect on the accompanying substances. These substances, split proteins, sugars, salts, etc., which are present in a malt extract in addition to amylases, apparently are prominent factors influencing amylase stability.

The dependence of amylase stability on the concentration of the malt extract assumes considerable importance even at near-room tem-

peratures. Figure 4 shows the influence of storage at 30°C and pH 5.0, with and without added calcium ion, on the stability of alpha-amylase in concentrated and dilute malt extracts. Both barley and wheat malt were used. The "concentrated" extracts were in the ratio of 1 part of meal to 10 parts of water. The "dilute" extracts were made by diluting these 1-to-10 extracts so that the ratio became equivalent to 1-to-250. Where calcium addition is indicated, the concentration was 1.0 mg per ml in the "concentrated" and 0.004 mg per ml in the "dilute" extracts. Data for beta-amylase activity are not given; at pH 5.0 the change in activity of this enzyme was inappreciable in either the concentrated or dilute extracts.

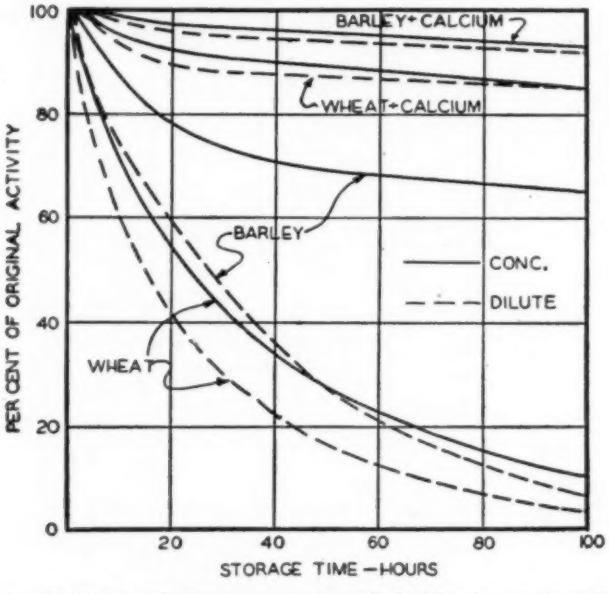

Fig. 4. The influence of extract concentration and of calcium ions on the stability of malt alpha-amylase at pH 5.0 and 30°C.

The curves of Figure 4 show that pronounced loss of alpha-amylase occurred when no calcium was added to the extracts, this loss being greater in the dilute than in the concentrated extracts. As in the previous studies barley-malt alpha-amylase was more stable than that from wheat malt. The addition of calcium ion greatly stabilized alpha-amylase and practically eliminated the differences between concentrated and dilute extracts. In the presence of added calcium some 93% of the barley-malt alpha-amylase remained active at the end of 96 hours and 85% of the wheat-malt alpha-amylase.

The data of Figure 4 have considerable significance in view of the fact that a pH of 5.0 is often approached in malt extracts. Too, most

methods developed for the measurement of alpha-amylase activity require a considerable dilution of the malt extract. It would seem that in processes utilizing the action of alpha-amylase, and certainly in methods designed for the measurement of this enzyme, preservation of its activity should be insured either by extracting with a solution of a calcium salt or by the addition of calcium to the extract. In this laboratory the practice has been to substitute a solution of either calcium chloride or calcium acetate, 2 mg of the salt per ml, for water in all malt extractions where alpha-amylase activity is a factor to be considered. It should be remembered that the addition of salts to the extraction medium, even in this low concentration, results in a greater yield of amylase in the extract. Accordingly, if the activity of a "water extract" is to be evaluated the calcium salt should be introduced immediately following the extraction.

Stability of the amylases at low pH: In the preceding studies experimental conditions were such as to be conducive to the inactivation of either beta-amylase alone or of both the alpha and beta components. A procedure usually considered to lead to the preferential inactivation of alpha-amylase involves low pH (in the region of pH 3.3) and low temperature. It has been observed on numerous occasions that whereas an acid treatment of malt extract resulted in a preferential destruction of alpha-amylase, the last traces of this enzyme could be removed only with difficulty. In fact a treatment severe enough to inactivate all the alpha-amylase invariably resulted in a pronounced loss of beta-amylase. The above data (Figs. 1 and 4) indicate that the calcium ion is a pronounced stabilizing factor for alpha-amylase. Other data (unpublished) indicate that the presence of calcium ion may contribute to the *instability* of beta-amylase. Accordingly experiments were carried out to determine the effect of the calcium ion on the relative stabilities of the amylase components at low pH with the object of devising a technique for achieving essentially complete inactivation of alpha-amylase coincident with maximum preservation of beta-amylase.

Table VI shows the results obtained by acid treatments of malt extracts. Distilled water extracts were made of wheat malt flour (1 g to 10 ml) and of barley malt meal (1 g to 20 ml). Previous to acidification the extracts were each divided into three parts; one part was used without further treatment, another was dialyzed for two days against running distilled water, and the third was dialyzed against running tap water for two days. Following these treatments the calcium contents of the three barley-malt extracts were 0.06 mg of calcium per milliliter for the original extract, 0.0015 mg for that dialyzed against distilled water, and 0.11 mg for the extract dialyzed against tap water. Dis-

tilled-water dialysis thus reduced the calcium content to one-fortieth of that of the original, whereas tap-water dialysis increased it to about twice that of the original. For the acidification treatments the pH of each extract was reduced to 3.3, held there for 60 minutes, and then brought up to pH 5.0 before activities were determined. The temperatures used for the treatments were 30°C for the wheat-malt extracts and ice water for those from barley malt.

TABLE VI

THE DIFFERENTIAL STABILITY OF THE MALT AMYLASES AT LOW PH AS INFLUENCED BY THE DIALYSIS MEDIUM

Malt	Dialysis	Temperature of treatment	Fraction of orig. activ. remaining after 60 min. pH 3.3	
			Alpha-amylase	Beta-amylase
			%	%
Barley	None	Ice water	0.75	96
Barley	Distilled water	Ice water	0.051	93
Barley	Tap water	Ice water	3.91	72
Wheat	None	30°C	0.17	98
Wheat	Distilled water	30°C	trace	100
Wheat	Tap water	30°C	2.42	63

It may be seen from the data of Table VI that dialysis of the malt extracts against tap water increased the stability of alpha-amylase and decreased the stability of beta-amylase. On the other hand, the alpha-amylase activities of those extracts dialyzed against distilled water were reduced to very low levels. This was coincident with almost complete preservation of beta-amylase activity. The extracts that received no dialysis treatment were intermediate with respect to the stability of alpha-amylase but did not differ appreciably in beta-amylase stability from those dialyzed against distilled water. The apparent difference between the stabilities of the barley and wheat malt alpha-amylases may be attributed to the difference in the temperatures used for the treatments. There appears to be a direct relationship between the stability of alpha-amylase at low pH and the calcium content of the extract.

Further evidence of the manner in which calcium is related to the stability of the two amylase components is presented in Figure 5. A wheat-malt extract (1 to 10) was dialyzed against distilled water for two days, then divided into four portions and calcium chloride added at the rate of 0.0, 0.05, 0.10, and 0.50 mg calcium per milliliter. The pH of each portion was then lowered to 3.25 and maintained there for 60 minutes at 30°C. At the end of this treatment the pH was adjusted to 4.5 with sodium acetate and activities determined.

The curves of Figure 5 indicate that the addition of calcium ion had a stabilizing effect on alpha-amylase up to 0.1 mg per ml; above this level no change was noted. The stability of beta-amylase decreased progressively with the addition of calcium at least up to the point of 0.5 mg addition. The "best" treatment (no calcium added) resulted in a product essentially free from alpha-amylase activity but retaining

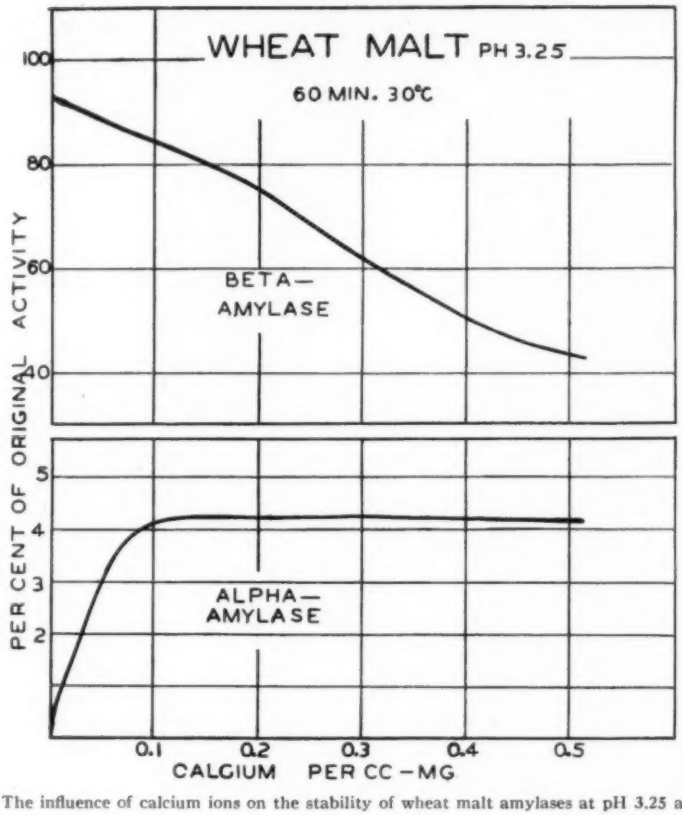

Fig. 5. The influence of calcium ions on the stability of wheat malt amylases at pH 3.25 and 30°C.

92% of the original beta-amylase activity. On the other hand, the product of the "worst" treatment (0.5 mg calcium added per ml) showed a retention of over 4% of the alpha-amylase and a loss of nearly 60% of the beta-amylase. Results very similar to those shown in Figure 5 were obtained when identical treatments were used with a barley-malt extract: the addition of 0.50 mg calcium per ml resulted in a product retaining 5% of the original alpha-amylase activity and only 58% of the original beta-amylase.

The opposite influences of calcium ion on the stabilities of the two malt amylase components further indicates a fundamental difference

in the nature of these two enzymes. In view of its stabilizing action on alpha-amylase activity it is remarkable that calcium is an *instability* factor for beta-amylase. No mechanism is apparent to explain the processes involved.

Temperature effects at low pH: Customarily acidification treatments of malt for differential inactivation of the amylases have been at low temperatures (ice water). The data of Table VI indicate that, with wheat malt, such treatments may be used at temperatures as high as 30°C without appreciably influencing the stability of beta-amylase. Table VII shows comparative data obtained when wheat- and barley-malt extracts, after two days of dialysis against distilled water, were treated for various time intervals at pH 3.3 at both ice-water and 30°C temperatures. One-to-10 wheat-malt and 1-to-20 barley-malt extracts were used; at the time these experiments were conducted it was considered advisable to balance the alpha-amylase concentrations in the extracts before treatment (the barley-malt meal had almost exactly twice as much alpha-amylase activity as did the wheat-malt flour).

TABLE VII

THE INFLUENCE OF TEMPERATURE ON THE DIFFERENTIAL INACTIVATION OF MALT AMYLASES AT LOW pH
(Fractions of original activity remaining)

Period of treatment	Wheat malt (1–10 extract)				Barley malt (1–20 extract)			
	Alpha-amylase		Beta-amylase		Alpha-amylase		Beta-amylase	
	Ice water	30°C	Ice water	30°C	Ice water	30°C	Ice water	30°C
min	%	%	%	%	%	%	%	%
15	0.39	0.31	100	100	0.12	0.06	98	97
30	0.36	0.22	100	100	0.10	0.0	92	96
60	0.35	trace	100	100	0.05	0.0	91	96
120	0.31	0.0	100	100	0.0	0.0	89	94

The data of Table VII show that at pH 3.3 alpha-amylase is inactivated more rapidly at 30°C than at ice-water temperature. Under the conditions used beta-amylase was as stable at 30°C as at ice water temperature.

Hydrogen-ion variation at low pH: The use of a hydrogen-ion concentration at or just below pH 3.3 for inactivation of alpha-amylase has been customary. However, the removal of most of the calcium ion from solution results in a condition such that beta-amylase activity survives treatments at considerably higher concentration of hydrogen ion. A 1-to-5 barley-malt extract was dialyzed against distilled water in the customary manner and portions then maintained at pH values

of 3.2, 3.0, 2.8, and 2.6 for 1 hour at 30°C. The results were as follows: at pH 3.2 a trace of alpha-amylase remained and 93% of the beta-amylase; at pH 3.0, no alpha and 92% of the beta; at pH 2.8, no alpha and 87% of the beta; at pH 2.6, no alpha and 53% of the beta. Thus it is apparent that a low concentration of calcium ion permits the use of lower pH. It would seem that with malt extracts dialyzed against distilled water a pH value of 3.0 for 1 hour at 30°C should serve to remove all the alpha-amylase activity with practically maximum "yield" of beta-amylase.

Influence of extract concentration at low pH: As in the thermal inactivation studies extract concentration was found to influence the stability of alpha-amylase at low pH. Following a two-day distilled-water dialysis of a 1-to-5 barley malt extract dilutions were made such that solutions essentially approximating 1-to-5, 1-to-10, and 1-to-20 could be compared. The hydrogen-ion concentration was adjusted to pH 3.0 in all cases and time intervals of 30, 60, and 120 minutes used for the treatments. It was found that alpha-amylase inactivation was dependent on extract concentration. Following 30 minutes' treatment an appreciable amount of alpha-amylase activity remained in the 1-to-5 extract, a trace in the 1-to-10, and none in the 1-to-20. In this instance a 1-hour treatment sufficed to inactivate all the alpha-amylase at any concentration. The fraction of beta-amylase remaining active was essentially constant throughout all treatments, varying between 90% and 93% of the original. Even under the most severe conditions (1-to-20 concentration for 2 hours) 92% of the beta-amylase survived.

These data indicate that a 1-hour treatment at pH 3.0 and 30°C is adequate to bring about maximum inactivation of alpha-amylase and minimum inactivation of beta-amylase in barley malt extracts, varying between 1-to-5 and 1-to-20 concentration. However, a 2-hour treatment resulted in no further loss of beta-amylase and a logical "safe" recommendation to insure complete inactivation of alpha-amylase could be to treat a dialyzed extract at pH 3.0 and 30°C for 2 hours.

Amylases of ungerminated cereals: In the past perhaps the most practical means of isolating beta-amylase relatively free from alpha-amylase has been to extract ungerminated cereals such as barley or wheat (Sandstedt, Kneen, and Blish, 1939; Ballou and Luck, 1941). However it is generally acknowledged that these cereals frequently contain small but variable amounts of alpha-amylase. In this laboratory it has been found that such amounts of alpha-amylase often are sufficient to interfere seriously with the use of wheat and barley as sources of "pure" beta-amylase. Accordingly, the procedure of acid inactivation subsequent to distilled-water dialysis evolved above for malt was applied to ungerminated wheat and barley.

TABLE VIII

THE INFLUENCE OF LOW pH ON THE ACTIVITY OF THE AMYLASES OF UNGERMINATED WHEAT

Extraction medium (18 hr.—30°C)	Treatment (1 hr.—30°C)	Alpha-amylase activity	Beta-amylase activity
		units	*units*
Water	None	0.061	10.9
NaCl	None	0.061	16.4
Water	pH 3.3	0.030	10.4
NaCl	pH 3.3	0.003	15.3
Water	pH 3.0	0.028	9.8
NaCl	pH 3.0	0.0	14.7

Table VIII shows the results of treating wheat extracts at pH 3.3 and 3.0, in each case for 60 minutes at 30°C. One to 5 extracts of wheat meal were dialyzed in the usual manner against distilled water for 2 days. Following dialysis the extracts were diluted so that they were equivalent to 1-to-10 concentrations. Two extractants were used, water and 5% sodium chloride, and in both cases the extractions were for 18 hours at 30°C in order to achieve a high concentration of beta-amylase.

The data of Table VIII show that a 5% sodium chloride extraction preceding dialysis and acidification can be used to advantage. A high level of beta-amylase was extracted and some 90% of this remained active even after the more severe treatment. At the same time alpha-amylase activity, while not completely removed at pH 3.3, was reduced to zero at pH 3.0. In contrast to the sodium chloride extract, that made with water showed some peculiarities. The beta-amylase activity was low throughout as might be anticipated and the alpha-amylase showed a surprising degree of stability. Even after the treatment at pH 3.0 nearly 50% of the alpha-amylase remained active. The difference between the two extracts with regard to beta-amylase concentration has been explained on the basis of protein peptization. The reason for the difference in stability of alpha-amylase *following* dialysis is obscure.

Table IX shows a comparison of various extracts of ungerminated wheat and barley when subjected to acid treatment. Three types of extraction were used with each cereal, all in 1-to-5 ratio for 18 hours at 30°C: a water extract, a 5% sodium chloride extract, and a water-papain extract (papain added at the rate of 5% of the meal). Following dialysis against distilled water the extracts were diluted to the equivalents of 1-to-10 concentrations, then treated at pH 3.0 for 1 hour at 30°C.

It may be seen in Table IX that with either barley or wheat the alpha-amylase of a water extract was remarkably stable. In contrast

the treatment resulted in almost complete inactivation of the alpha-amylase in the papain extracts and complete inactivation in the sodium chloride extracts. Again the reason for these differences is obscure but it might be postulated that protein peptization either by the salt or by papain may contribute to the instability of alpha-amylase. With all extracts the beta-amylase was stable, its activity being preserved to the extent of over 90%.

TABLE IX

ACID INACTIVATION OF THE ALPHA-AMYLASE IN VARIOUS EXTRACTIONS OF UNGERMINATED WHEAT AND BARLEY

(Fractions of original activity remaining after treatment—pH 3.0, 1 hour, 30°C)

Extractant	Wheat		Barley	
	Alpha-amylase	Beta-amylase	Alpha-amylase	Beta-amylase
	%	%	%	%
Water	59	91	97	93
5% NaCl	0	96	0	95
5% papain	5.4	94	2.4	91

It appears from the above that a sodium chloride extract is a good starting material for the preparation of beta-amylase free from the alpha component. As with the malts it could be recommended that the acid treatment be extended to 2 hours at 30°C. This was done with the dialyzed sodium chloride extracts and, as with the 1-hour treatments, it was found that 96% of the wheat beta-amylase activity and 95% of that originally in the barley were retained, coincident with complete inactivation of the alpha-amylase.

Methods for the Isolation of the Malt Amylases

The following methods have been used repeatedly in this laboratory and have proved consistently adequate.

Alpha-amylase: Either barley or wheat malt is used as a starting material. The finely ground meal is extracted in 1g-to-5ml ratio for 1 hour at 30°C with a solution containing 2 mg per ml of either calcium chloride dihydrate or calcium acetate. The pH of the extract is adjusted to approximately 6.0 with either sodium hydroxide or sodium acetate solution, following which the temperature of the extract is raised to 70°C and held there for 15 minutes. After cooling and filtering the extract may be used without further treatment, or the alpha-amylase may be concentrated by appropriate salt or alcohol precipitation techniques.

Beta-amylase: Barley or wheat, either malted or not and either as the whole meals or as flours, may be used as starting materials. In this laboratory the use of either ungerminated barley or barley malt

has been attended by least complications; accordingly, an ungerminated barley of high beta-amylase activity customarily is used. The finely ground meal is extracted in 1g-to-5ml ratio at 30°C with 5% sodium chloride for from 3 to 18 hours, as convenient. The extract is dialyzed against cool (approx. 20°C) running distilled water for two days. The pH of the extract is then lowered to 3.0 with 1.0*N* hydrochloric acid and held at that pH for 2 hours at 30°C. Following this acidification treatment, the pH is raised to between 4.5 and 5.0 by the appropriate addition of sodium hydroxide (0.1*N*) or sodium acetate (8%) solution. The solution is then ready for use by itself, or the beta-amylase may be concentrated by appropriate salt or alcohol precipitation techniques.

Use of beta-amylase in the determination of alpha-amylase activity: The alpha-amylase method of Sandstedt, Kneen, and Blish (1939) requires a special substrate resulting from the treatment of soluble starch with an excess of "pure" beta-amylase. These authors recommended the addition of 5 ml of a 2g-to-5ml hard-wheat-flour extract per 500 ml of 2% starch, the resulting substrate to be used not less than 24 nor more than 48 hours thereafter. Further work confirms their finding that the above level is adequate to cover any normal range of variation in hard-wheat flours.

It was found that when an acid treated extract from barley meal was diluted to an equivalent of 1g-to-40ml concentration, 4 ml of this dilution per 100 ml of starch resulted in a substrate ready to use in 18 hours and good for at least 66 hours. (The beta-amylase activity of this extract was 13.2 units per gram of original barley meal extracted.) Obviously then one-eighth of the volume of beta-amylase solution used, *i.e.*, 0.5 ml per 100 ml of starch, would be an adequate addition of the original 1-to-5 solution. Since the beta-amylase activities of solutions prepared by acid treatment of 1-to-5 extracts of barley or barley malt rarely, if ever, are less than one-half that of the above, a reasonably safe addition would consist of 1 ml per 100 ml of starch, the substrate to be used not less than 18 hours thereafter. Such a substrate apparently retains its utility for some two to three days but does deteriorate on long standing.

If it is desirable to have a more accurate calculation of the amount of beta-amylase solution to be added, the following procedure may be employed: Calculate the beta-amylase activity of the solution to be used in units per milliliter, *i.e.*, the number of grams of starch hydrolyzed by *1 ml* of solution in 1 hour at 30°C (Kneen and Sandstedt, 1941). Then 1.65 divided by this figure is the number of ml of the solution which will provide 20% more beta-amylase than is essential for conversion of the starch to the desired substrate by 18 hours' action at 30°C.

It should be noted that beta-amylase prepared from barley or wheat is applicable in the preparation of a substrate for the determination of *cereal* alpha-amylase activity. The advisability of its use for the determination of the activity of other "alpha-amylases" such as those of animal or microbial origin has not been established and is not recommended.

Summary

The experiments reported in this communication dealt with the differential response of the malt amylases to the precipitation influence of ammonium sulfate and of alcohol and with the stability of these amylases to widely varied combinations of temperature, hydrogen-ion concentration, calcium-ion concentration, and extract dilution. Both wheat and barley malts were used throughout and in certain low-pH stability studies ungerminated wheats and barleys were employed.

The maximum precipitation of the beta-amylases of wheat and barley malts and the alpha-amylase of wheat malt occurred in the range of 25% to 35% ammonium sulfate concentration. Barley-malt alpha-amylase showed maximum precipitation in the 15% to 25% range of salt concentration. Both the alpha- and beta-amylases of barley malt were precipitated by somewhat lower salt concentrations than the corresponding amylases of wheat malt.

The maximum precipitation of the beta-amylases of wheat and barley malts occurred in the range 56% to 68% alcohol concentration. Wheat-malt alpha-amylase showed maximum precipitation in the range 50% to 56% alcohol and barley-malt alpha-amylase in the range 44% to 50% alcohol concentration. Here too, the barley-malt amylases were precipitated at somewhat lower concentrations of precipitating agent than the corresponding amylases from wheat malt. Because of over-lapping solubilities, no clear-cut separation of the two amylase components could be effected by the precipitation techniques.

The differential inactivation studies at relatively high temperature, 50° to 70°C, combined with hydrogen-ion concentrations between the pH values 4.0 to 7.0, demonstrated that the stability of either amylase was influenced by both these factors. For example, with a given temperature treatment the stability of beta-amylase was dependent on the pH of the solution and at a given pH the stability of alpha-amylase was dependent on the temperature used. However, temperature was the more significant factor with beta-amylase, hydrogen-ion concentration with alpha-amylase.

A factor of prime importance bearing on the stability of the amylases was the calcium-ion content of the solutions. Calcium proved to be a *stability* factor for alpha-amylase, and an *instability* factor for

beta-amylase. This differential effect on stability was utilized in devising methods for the preparation of the two amylases: Conditions for maximum retention of alpha-amylase (90% to 100%) and complete inactivation of beta-amylase were the *presence* of calcium, relatively high pH (6.0 to 7.0), and a temperature of 70°C. Conversely conditions for maximum retention of beta-amylase with complete inactivation of alpha-amylase were found to be the *absence* of calcium, relatively low pH (3.0), and a temperature of 30°C.

Another factor of importance was the extract concentration: the more dilute the extract, the less stable the amylases. This instability appeared to be dependent not on the amylase concentration but on the concentration of accompanying substances in the extract.

In addition to the variation in response to alcohol and salt precipitation, a marked difference was noted between the amylases of wheat and barley malts with respect to thermostability. Whereas barley-malt alpha-amylase was more stable to heat than wheat-malt alpha-amylase, just the reverse was found for the beta components; beta-amylase from wheat malt was more stable than that from barley malt.

Methods found to be practical for the preparation of alpha-amylase free from beta and beta-amylase free from alpha are presented. In addition the technique of using such "pure" beta-amylase preparations in the preparation of the substrate for the determination of alpha dextrinogenic activity is outlined.

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COMMERCIAL WHEAT GERM, ITS COMPOSITION[1]

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Wheat germ is fast becoming a popular article of diet, and scores of mills are actually separating the germ and selling it as such. As the germ constitutes about 2% of the wheat kernel, the total potential output of this product from the milling of 110 million barrels of flour amounts to some 300,000 tons annually. In practice, however, the miller probable does not recover more than one-fourth of the total amount in the grain.

General Discussion

A number of investigators have published data on the composition of wheat germ. Richardson (1884) reports the following data as the

[1] Agricultural Chemical Research Division Contribution No. 69.

average of three samples: moisture 8.37%, ash 4.60%, fat 12.51%, crude fiber 1.49%, and protein 30.08%. These results are in close agreement with those obtained by later investigators except that the crude fiber has been found slightly lower. Forbes, Beegle, and Mensching (1913) reported the mineral content of wheat germ. Sullivan and Near (1927) reported 25 ppm of aluminum as well as 420 ppm of zinc in wheat germ. Richardson and Crampton (1886) isolated from the germ portion of wheat allantoin and a nonfermentable sugar which was later identified as raffinose by Schulze and Frankfurt (1895); the latter (1896) reported that wheat germ contains 6.89% of raffinose. Schulze and Frankfurt (1893) showed conclusively that germ contained betaine and choline. They reported a yield of 0.16% to 0.2% of betaine chloride, but considerably less choline. Sitosterol from wheat germ was first isolated by Burian (1897). Powers and Salway (1913) isolated sinapic acid from wheat germ and confirmed the findings of others regarding the presence of sitosterol, choline, betaine, allantoin, cane sugar, dextrose, and raffinose. Sullivan and Howe (1937) isolated glutathione from wheat germ, the yield being 0.1 to 0.2 g from 2 kg of material. Andrews and Bailey (1932) made a study of the distribution of the organic phosphorus in wheat and wheat products. They reported the total phosphorus in the germ as 1.244%, of which 47.98% was in the form of phytin, 5.70% lipoid, and 46.3% undetermined. Frankfurt (1896) found that wheat germ contained 1.55% of lecithin. Osborne and Mendel (1919) found 7.5% of dextrin and 8.29% of pentosans in wheat germ. Kalning (1918) reported a yield of 11.55% of pentosans. In addition to the various constituents mentioned, wheat germ may contain from 7,000 to 9,000 μg of vitamin B_1 per pound. Besides being an excellent source of this vitamin, it is appreciably rich in vitamins A and B_2 and likewise is one of the chief sources of vitamin E.

Soft red winter, hard red winter, hard red spring, durum, and white are the five important classes of wheat grown in the United States. Nineteen samples of germ from these five classes of wheat were secured, all having been commercially milled. In a preliminary investigation several samples of germ from the same mill were used in baking tests and it was found that they differed appreciably in their bread-making properties. Most if not all samples of commercial germ subsequently studied contained more or less bran and endosperm, some being contaminated largely with bran, some with both bran and flour, and others chiefly with endosperm or flour. The investigation here reported was undertaken to study the composition of germ from different classes of wheat and the behavior of the different germ samples when used in baking. How commercial germ varies in composition will be shown below.

Investigations

Determinations and methods: The constituents and chemical characteristics, including moisture, protein, nonprotein nitrogen, crude fiber, fat, furfural (in a few samples only), total sugar, diastatic power, ash, calcium, magnesium, potassium, phosphorus, iron, copper, manganese, chlorine, and sulfur were determined according to the methods of the Association of Official Agricultural Chemists (1935). The results are given in Tables I to V. The terms "nitrogen-free extract" and "carbohydrates by difference" are frequently used to designate the undetermined fraction. In the case of many cereal products this is chiefly starch.

Proximate composition: The average moisture content of germ (9.2%) is considerably less than that of flour, which averages about 13.0%. This low moisture content of germ enhances its keeping quality. If the germ is protected from undue exposure to light and is kept at a temperature not much above 50°F it will keep fresh for several months. The protein content of the 19 germ samples varied from 18.3% to 35.3% and averaged 28.9%; this average is more than twice that for the protein content of flour. The ash content averaged 4.1%. The percentage of sugar (mostly sucrose) averaged 13.7%, the minimum being 6.8% and the maximum 17.4%. In general, the fiber content of germ averaged about the same as that of whole wheat. The fat content averaged 9.7% with a maximum of 15.0% and a minimum of 5.2%, indicating in the latter case considerable contamination with flour.

None of the 19 samples of commercial germ studied was pure. On the contrary, each was contaminated to some extent with flour or bran, but particularly with flour.

The amount of the undetermined carbohydrates present in commercial germ is probably the best index of the degree of purity in most cases. The average figure for these carbohydrates was 32.33%, the maximum being 53.0% and the minimum 19.18%. The germ fractions from hard red spring and durum wheats were the lowest in these carbohydrates, the average for the two types being 24.3%, as against 30.4% for hard red winter, 36.0% for white wheat, and 39.8% for soft red winter wheat. These figures indicate that the endosperm or flour portion is more easily separated from the germ in the case of the hard wheats than in the case of the soft wheats. This may be because the endosperm of the hard wheats is more granular in form than the endosperm of the soft wheats. The results of this study, however, seem to justify the conclusion that pure germ from hard wheats is appreciably higher in protein content than is pure germ from the

TABLE I

PROXIMATE COMPOSITION OF WHEAT GERM

Lab. No.	Moisture	Protein (N ×6.25)	Fat ether extract	Ash	Total sugar calculated as invert	Crude fiber	Other carbohydrates by difference
	%	%	%	%	%	%	%
WHITE							
1648	9.1	25.1	9.2	3.6	15.1	1.8	35.7
1657	8.4	25.5	9.4	3.5	15.1	2.4	35.7
1660	8.3	24.6	9.1	3.5	14.9	3.0	36.7
Av	8.6	25.2	9.2	3.5	15.0	2.4	36.0
SOFT RED WINTER							
1662	8.9	34.4	10.6	4.9	15.7	1.7	23.9
1646	9.6	28.4	9.3	4.4	12.7	1.9	33.7
1647	9.9	27.0	7.4	4.5	11.1	2.9	37.2
1645	9.8	25.1	7.2	3.5	11.6	1.7	41.1
1656	10.4	20.4	5.9	3.1	8.9	1.7	49.7
1669	11.5	18.3	5.2	3.4	6.8	1.9	53.0
Av	10.10	25.6	7.6	3.9	11.1	1.9	39.8
HARD SPRING							
1652	8.7	32.7	10.7	4.1	14.6	1.6	27.7
1653	8.8	35.3	12.4	4.8	17.4	1.7	19.8
1654	8.5	33.6	12.4	4.6	16.5	1.8	22.7
1668	10.6	30.7	10.9	4.1	15.1	1.8	26.9
Av	9.2	33.1	11.6	4.4	15.9	1.8	24.3
HARD RED WINTER							
1649	8.9	33.4	11.2	4.5	14.3	1.8	25.9
1650	9.9	27.7	7.2	3.8	10.7	2.9	37.8
1651	9.3	32.4	9.7	4.5	13.4	2.3	28.5
1661	8.6	31.3	9.5	4.5	15.1	1.7	29.4
Av	9.2	31.2	9.4	4.3	13.4	2.2	30.4
DURUM							
1658	8.8	29.7	11.9	4.8	15.0	3.5	26.3
1655	7.4	32.7	15.0	4.5	15.8	2.1	22.2
Av	8.1	31.2	13.5	4.6	15.4	2.6	24.3
Minimum	7.4	18.3	5.2	3.1	6.6	1.6	19.2
Maximum	11.5	35.3	15.0	4.9	17.4	3.5	53.0
Average	9.2	28.9	9.7	4.1	13.7	2.1	32.3

TABLE II

The Nitrogen Fractions of Wheat Germ—Water-Free Basis

Lab. No.	Total N	70% alcohol sol N	3% $NaCl$ sol N	5% K_2SO_4 sol N	$Cu(OH)_2$ pptd N	Glutenin N[1]	Albumin and globulin[2]	N not pptd by phosphotungstic acid	N not pptd by $Cu(OH)_2$
	%	%	%	%	%	%	%	%	%
WHITE									
1648	4.4	0.6	2.9	2.7	4.1	1.2	2.4	0.6	0.4
1657	4.4	.6	2.9	2.6	4.1	1.2	2.3	.6	.3
1660	4.3	.6	2.9	2.6	3.9	1.1	2.3	.6	.3
Av	4.4	.6	2.9	2.6	4.0	1.2	2.4	.6	.3
SOFT RED WINTER									
1662	6.0	.7	4.3	3.7	5.2	1.9	3.4	.9	.8
1646	5.0	.6	3.1	2.6	4.6	1.8	2.4	.7	.4
1647	4.8	.7	3.0	2.6	4.4	1.6	2.3	.7	.4
1645	4.5	.7	3.0	2.5	4.2	1.3	2.4	.7	.3
1656	3.6	.7	1.9	1.9	3.4	1.0	1.4	.4	.3
1669	3.3	.7	1.9	1.6	3.1	1.0	1.6	.4	.3
Av	4.5	.7	2.9	2.5	4.1	1.4	2.4	.6	.4
HARD SPRING									
1652	5.7	.8	3.9	3.5	5.1	1.4	3.1	.8	.6
1653	6.2	.7	4.5	3.9	5.1	1.6	3.6	.9	.6
1654	5.9	.7	4.3	3.7	5.3	1.5	3.4	.9	.6
1668	5.5	.8	3.7	3.1	4.9	1.6	2.8	.9	.6
Av	5.8	.8	4.1	3.6	5.2	1.5	3.2	.9	.6
HARD RED WINTER									
1649	5.7	.7	3.2	3.0	5.1	1.9	2.4	.9	.6
1650	4.8	.8	3.3	2.6	4.2	1.4	2.6	.7	.5
1651	5.6	.8	3.9	3.3	4.9	1.6	3.1	.8	.8
1661	5.6	.7	3.9	3.3	4.9	1.5	3.1	.7	.6
Av	5.4	.8	3.7	3.1	4.8	1.6	2.8	.8	.6
DURUM									
1655	5.7	.7	2.8	2.9	4.9	2.1	2.1	.7	.7
1658	5.2	.7	3.5	2.9	4.6	1.6	2.9	.7	.6
Av	5.5	.7	3.2	2.9	4.8	1.9	2.5	.7	.7
Minimum	3.3	.6	1.9	1.6	3.1	1.0	1.4	.4	.3
Maximum	6.2	.8	4.5	3.9	5.6	2.1	3.6	.9	.8
Av all samples	5.1	.7	3.3	2.9	4.6	1.5	2.6	.7	.5
FLOUR[3]									
Av	2.3	1.2	0.6	0.3	2.0	0.8	0.5	.1	.1

[1] Total nitrogen minus values for 5% potassium sulfate soluble nitrogen and 70% alcohol soluble nitrogen.

[2] Three percent sodium chloride soluble nitrogen minus nitrogen not precipitated by phosphotungstic acid.

[3] Average of 4 samples.

soft wheats. Pure germ from durum wheat is probably higher also in fat content than is that from the other classes of wheat.

The nitrogen fractions of wheat germ: The proteins of wheat are important because of their nutritional value and also because of the role they play in imparting bread-making properties to the flour. Both phases have been the subjects of numerous investigations which, however, have hitherto been confined chiefly to wheat and flour.

The proteins of flour were formerly regarded as having properties which would place them in distinct groups, depending on their behavior in certain solvents. In recent years there has been a change of opinion with regard to the value of solubility data for classifying proteins. Gortner, Hoffman, and Sinclair (1929) found that the amount of protein extracted by salt solutions depended on the nature and strength of the solution used and not on the presence of definite simple proteins in the flour; from this work it appears that the "protein solubility" is not "solubility" in the strict sense of the term, but is peptization; and that the so-called "simple proteins" are not distinct individuals. but in reality are heterogeneous mixtures.

The protein fractionations were likewise made according to the methods of the A. O. A. C. and consisted in extracting the germ: (1) with 70% alcohol, (2) with 3% sodium chloride, and (3) with 5% potassium sulfate. In addition to the fractions extracted by these solvents (generally used with flour), the total protein nitrogen precipitated by cupric hydroxide was determined, as well as the total protein nitrogen precipitated by a 20% solution of phosphotungstic acid, and the nonprotein nitrogen was estimated by subtracting from the total nitrogen the nitrogen precipitated by copper or phosphotungstic acid. The data appear along with total nitrogen in Table II. Calculations were made on the dry basis.

Three-percent sodium chloride was used instead of 1% because the Protein and Nutrition Division of the Bureau of Agricultural and Industrial Chemistry has found that the higher concentration gives more nearly pure albumin and globulin than does the more dilute salt solution. In making these separations the samples were allowed to remain in contact with the solvents overnight at 50°F instead of 3 to 4 hours as recommended by the official methods. To make possible a comparison with flour, four samples of flour were subjected to the same treatments. The average results obtained appear in Table II.

In Table III are shown the average values for total nitrogen and for the various nitrogen fractions in germ samples of high and low carbohydrate content, respectively. The eight samples of germ containing the largest amounts of carbohydrates (average 40.9%) have

an average of 4.29% of nitrogen. The eight samples with lowest carbohydrates (average 24.4%) have an average nitrogen content of 5.73%.

The 70% alcohol-soluble nitrogen is the fraction which, in the case of flour, is usually referred to as gliadin. From the averages of results on all samples it appears that wheat germ contains over twice as much total nitrogen as does spring wheat flour (5.06% and 2.32%, respectively). However, the germ contains only a little more than half as

TABLE III

AVERAGE VALUES SHOWING THE RELATIVE PROTEIN FRACTIONS OF TOTAL N IN WHEAT GERM SAMPLES OF HIGH AND LOW CARBOHYDRATE CONTENTS

Classification of samples as to proportion of carbohydrate	Total N %	70% alcohol sol N	3% NaCl sol N	5% K_2SO_4 sol N	$Cu(OH)_2$ pptd N	Albumin and globulin N	N not pptd by phosphotungstic acid	N not pptd by $Cu(OH)_2$	Glutenin N
	percent of total nitrogen								
High in carbohydrate (8 samples; av 40.8) [1]	4.29	15.4	63.8	55.9	92.0	50.2	13.6	8.0	28.7
Low in carbohydrate (8 samples; av 24.4) [2]	5.73	12.6	65.7	58.2	88.8	51.7	14.0	11.2	29.2
Av individual results on all samples (19)	5.07	13.9	65.1	57.0	90.2	51.4	13.7	9.8	29.1
Wheat flour (4 samples) (for comparison)	2.32	52.6	24.1	14.7	95.7	20.6	3.5	4.3	32.7

[1] The 8 samples highest in undetermined carbohydrates consisted of 4 of SRW, 3 samples of W, and 1 sample of HRW wheat.

[2] The 8 samples low in carbohydrates consisted of 4 samples of HRS, 2 samples of Durum, 1 sample of HRW, and 1 sample of SRW wheat.

much nitrogen soluble in 70% alcohol as does the flour (0.69% and 1.22%, respectively). In other words, while less than 15% of the germ nitrogen is soluble in the alcohol solution, over 50% of the flour nitrogen is thus soluble. In the case of the salt-soluble and K_2SO_4 soluble nitrogen, however, the reverse is true; the germ nitrogen averages *over* 60% soluble in the salt solution and *nearly* 60% soluble in the K_2SO_4 solution, whereas flour averages nearly 25% and 15% respectively.

The nonprotein nitrogen of wheat includes the nitrogen of amino acids, amides, certain nitrogen bases and lecithin. Teller (1932) claims that arginine and choline are present in wheat. Some of these nonprotein nitrogen compounds play an important role in nutrition.

The mineral content of wheat germ: Composite samples were prepared from each of the different classes of wheat. The samples selected were those which, according to chemical analyses, appeared to be most nearly pure germ. The results are given in Table IV. The oxides of

potassium, magnesium, calcium and phosphorus averaged 26.35%, 12.5%, 1.76% and 56.4%, respectively. These results indicate that the composition of the ash of wheat germ is not far different from that of the endosperm.

TABLE IV

MINERAL CONTENT OF WHEAT GERM

Class of wheat represented	Samples used per composite	Ash	Mg	Ca	K	P	Mn	Cu	Fe	Total oxides % of total ash	Cl	S
	No.	%	%	%	%	%	%	%	%	%	%	%
White wheat	3	3.57	0.264	0.048	0.798	0.851	0.0227	0.00125	0.0057	95.9	0.0852	0.167
Soft red winter	3	4.22	0.288	0.051	0.938	1.043	0.0237	0.00125	0.0041	95.4	0.078	0.246
Hard spring	4	4.36	0.329	0.051	0.913	1.122	0.0155	0.00150	0.0058	98.5	0.089	0.246
Hard winter	3	4.46	0.347	0.063	0.984	1.122	0.0227	0.00200	0.0064	98.9	0.078	0.262
Durum	1	4.67	0.380	0.063	1.015	1.135	0.0181	0.00263	0.0059	96.6	0.078	0.256
Av for 5 classes		4.26	0.322	0.055	0.930	1.055	0.0208	0.00175	0.0058	97.0	0.082	0.235
Flour[1] (for comparison)		0.48	0.030	0.018	0.055	0.116	0.0002	0.0002	0.0008	—	—	—

[1] Sullivan and Near, Ind. Eng. Chem. 19: 498, 1927.

Furfural: Furfural was determined on five samples of germ, one from each class of wheat. Those samples which seemed to be more nearly pure germ according to appearance and chemical analyses were used for the test. The results obtained were 4.96% furfural for the white wheat germ (a composite of three samples), 3.61% for the soft red winter (sample 1662), 3.62% for hard spring (a composite of two samples), 3.48% for hard red winter (sample 1649) and 3.66% for the durum wheat germ (sample 1658). The average for the germ samples from the five classes of wheat was 3.87%.[2] Under the conditions of the method used, pentoses, uronic acids, pectic substances, and hemicelluloses are the constituents which yield furfural.

Miscellaneous tests: The samples of wheat germ were all examined for organoleptic evidence of contamination (appearance and feel) and tested for weight per definite volume, diastatic activity, and influence on baking quality of flour. The data appear in Table V. Most of the samples were flaky, indicating that they had been prepared in the normal process of milling wheat into flour. From the data it is evident that it is quite impossible to estimate the degree of contamination of samples of germ by other wheat milling products or by-products from ocular observation or by feel. Three samples of germ which showed "very little" or "insignificant" contamination (judging from their

[2] This corresponds to 6.61% pentosans.

TABLE V

Properties and Proximate Composition of Wheat Germ—Miscellaneous Data

Lab No.	Apparent degree contamination by other wheat milling products or by-products; appearance and feel	Weight per cup	Diastatic activity as maltose figure[1]	Protein[2]	Fat[2]	Undetermined carbohydrates[2]
		g	*mg*	%	%	%
	WHITE					
1648	Insignificant; soft talcy feel	79	455	25.1	9.2	35.7
1657	Very little; yellow, soft talcy feel	78	460	25.5	9.4	35.7
1660	Very little; fine, soft feel	82	560	24.6	9.1	36.7
Av		79.7	492	25.2	9.2	36.0
	SOFT RED WINTER					
1662	Very little; coarse, talcy feel	70	405	34.4	10.6	23.9
1646	Moderate; soft granular feel	94	350	28.4	9.3	33.7
1647	Much	74	340	27.0	7.4	37.2
1645	Moderate; soft feel	74	542	25.1	7.2	41.0
1656	Much; light in color due to flour, very soft	72	468	20.4	5.7	49.7
1669	Very much; looks like feed middlings	76	315	18.3	5.2	53.0
Av		76.7	403	25.6	7.6	39.8
	HARD SPRING					
1652	Moderate; fairly soft	98	358	32.7	10.7	27.7
1653	Moderate; fairly soft	84	330	35.3	12.4	19.8
1654	Moderate; large flakes, some bran	79	375	33.6	12.4	22.7
1668	Very little; gritty	86	375	30.7	10.9	28.9
Av		86.8	360	33.1	11.6	24.3
	HARD RED WINTER					
1649	Very little; fine, granular feel	117	275	33.4	11.2	25.9
1650	Much	65	375	27.7	7.2	37.8
1651	Moderate; fairly soft	81	366	32.4	9.7	28.5
1661	Very little; yellow, coarse rough feel	82	480	31.3	9.5	29.4
Av		86.3	374	31.2	9.4	30.4
	DURUM					
1658	Little; soft feel	71	453	29.7	11.9	26.3
1655	Insignificant; very harsh and granular	143	330	32.7	15.0	22.2
Av		107	391	31.2	13.5	24.2
	Minimum	65	275			
	Maximum	143	560			
	Average	84.5	401			

[1] From 10 g germ.
[2] From Table I.

relatively low nitrogen contents) were in fact moderately or even much contaminated with endosperm. Moreover it is true that four of the six samples described as showing "moderate" contamination were actually considerably contaminated with endosperm. Of the 19 samples of wheat germ, two were granular in form and were considerably heavier than the flaked germ, the weight of one cupful or half-pint being 117 and 143 grams, respectively; the average weight of the other 17 samples was 79.1 grams, but even among these the weight varied from 65 to 98 grams.

The two forms of wheat germ—flaked and granular—are obtained by following quite different milling procedures. The flaked germ, as already stated, was produced in the regular process of milling; the germ particles in passing between the rolls became flattened. The granular germ probably resulted from removal of the germ by means of a gravity separator before the mixture went to the final rolls. These two procedures seem to be in extensive use in the milling industry for the manufacture of these two types of wheat germ.

While the total nitrogen of the two forms of wheat germ is essentially the same, the proportion of total N soluble in 3% salt solution is approximately 50% in the case of granular germ, as against 70% in the case of flaky germ. Likewise the proportions of total N soluble in K_2SO_4 are 50% and 60%, respectively.

Diastatic activity: The diastatic activity expressed as "maltose figure" was determined according to the Official Methods of the A. O. A. C. (1935) (Blish and Sandstedt). An average of 401 mg of maltose per 10 g of germ was found for the 19 samples, the maximum being 560 and the minimum 275. The samples of germ from white wheat yielded an average of 492 mg of maltose per ten grams of germ; those from soft red winter wheat germ, 403; durum, 391; hard red winter, 375; and hard red spring, 360 mg (see Table V). Commercial germ from soft red winter wheats averaged appreciably higher in maltose figure than did germ from the hard red wheats, in spite of the fact that the former is apparently more contaminated with flour than is the latter.

Summary

Nineteen samples of wheat germ were used in this investigation, including two to six samples from each of the five classes of wheat grown in this country.

Judging from composition, it would appear that the samples of germ from hard wheat were purer than those from soft wheat; *i.e.*, the former were freer from flour particles. The germ from soft red winter wheat contained an average of 39.79% of undetermined carbohydrate,

while the germ from hard spring wheat contained only 24.28%, the difference being largely due to admixed endosperm.

The germ from white wheat showed greater variations in its composition than that from the other classes. The diastatic activity and sugar were higher and the protein and ash lower in the germ from white wheats than in that from the other wheats.

Germ from hard red spring and durum wheats averaged appreciably higher in fat than did germ from winter wheats.

In general, the nitrogen content of the germ is correlated with the class of wheat; *e.g.*, hard spring wheat germ was highest in nitrogen, the average being 5.82%; durum wheat germ came next with 5.45%; then followed in descending order germ samples from hard red winter (5.42%), soft red winter (4.54%), and white wheat (4.38%).

The alcohol-soluble nitrogen of wheat germ makes up about one-seventh of the total nitrogen, whereas in flour fully half of the total nitrogen is alcohol-soluble. The salt-soluble nitrogen of wheat flour constitutes one-fourth of the total nitrogen, while that of the germ makes up over 60% of the total. The fraction regarded as albumin and globulin was found to be much higher in the germ than in the flour; it represented 51.2% of the total nitrogen in germ and 20.7% in flour.

In general, when the amount of endosperm was high the proportions of nitrogen soluble in 70% alcohol and the copper-hydroxide-precipitated nitrogen were high, whereas the proportions soluble in 3% sodium chloride and in 5% potassium sulfate solutions were low.

The average weight per unit volume of flaked germ was considerably less than that of granular germ.

Five composite samples of germ, each representing a different class of wheat, were analyzed for mineral content.

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WHEAT GERM IN BREAD MAKING[1,2]

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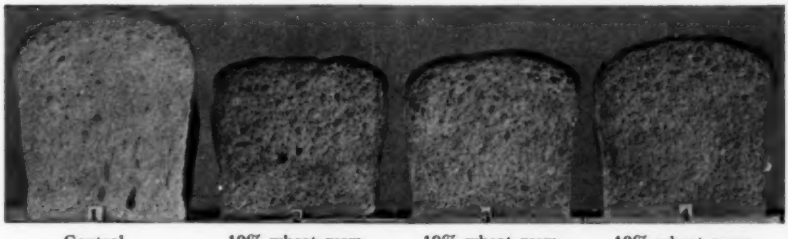
Much interest in the use of wheat germ as a food and in bread making has recently become apparent. About 30 years ago the importance of vitamins in the diet was first brought forth, and since that time tremendous progress in research on the vitamins has been made. Wheat germ has for some time been recognized as an excellent source of vitamin E, as well as a rich natural source of several of the B vitamins. The germ is very rich in most of the essential food factors present in the grain.

Bread furnishes practically as many calories in the diet as any other single article of food. Increasing the vitamin and mineral contents of bread and supplying good-quality protein through bread would insure much better nutrition to most people, particularly those in lower-income groups. This article reports the results of studies of the use of wheat germ in the baking of bread.

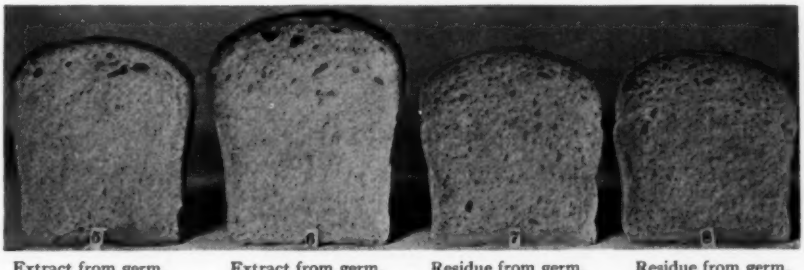
[1] Agricultural Chemical Research Division Contribution No. 89.
[2] Read by title at the annual meeting of the American Association of Cereal Chemistry, New York, N. Y., May, 1940.

Experimental

Effect of the whole germ, the extract, and the residue in bread: The basic formula and procedure adopted by the American Association of Cereal Chemists (1928) were used in making bread, except that a portion of the flour was replaced by germ. Two series were used, one in which the germ was merely wetted and the other in which the germ was first steeped or macerated in water for 18 hours at 10°C. (The term "steeping" as used in this paper applies to the soaking of the germ in water for a period of time varying from a half hour to 24 hours.) Figure 1 shows photographs of the bread.



Control (flour only) | 10% wheat germ, added dry | 10% wheat germ, wetted but not steeped | 10% wheat germ, steeped 18 hrs.



Extract from germ, wetted but not steeped | Extract from germ, steeped 18 hrs. | Residue from germ, wetted but not steeped | Residue from germ, steeped 18 hrs.

Fig. 1. The effect of steeped wheat germ, extract, and residue from steeped germ on bread-making properties (10% germ).

Loaf 4, which contained the steeped germ, was much better in texture, grain, volume, and color than Loaves 2 and 3. Loaf 5, which contained the extract from the germ which had simply been wetted, was not nearly so good as Loaf 6, which contained the extract from the germ steeped 18 hours. Loaf 6 was even better and lighter in color than the control. The marked increase in the whiteness of Loaf 6 as compared with that of the control is regarded as due to an increase in net oxidizing activities developed during the steeping of the germ. Loaf 7, which contained the residue left after extraction of the germ momentarily wetted with water, was slightly better than Loaf 8, which

contained the residue from the germ which had been macerated 18 hours. It is very evident that during the steeping of the germ changes take place which affect its bread-making properties. It is also evident that the substances which favorably affect the loaf are to be found in the extract rather than in the residue.

Effect of time of steeping on bread making properties: Bread doughs containing 15% of germ steeped 1, 2, 3, 5, and 7 hours were baked and compared with one that contained the germ which had simply been wetted. These breads were made according to the following formula and procedure:

Mix the following ingredients and let the mixture stand the required time (stated above).

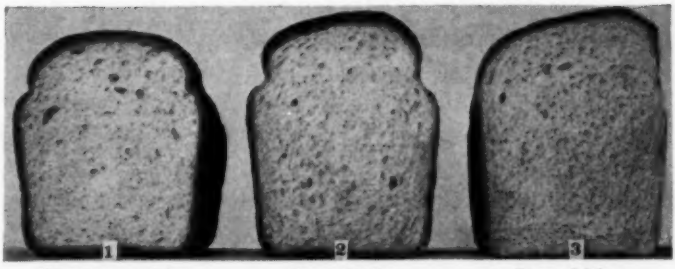
	Germ	47 g
	Salt	4.7 g
	Water	80 ml
	Potassium bromate	10 mg
Sponge:		
	Flour	180 g
	Water	100 ml
	Yeast	7.8 g
	The ingredients listed above	
	Allow to ferment 3 hours at 27°C.	

Dough: Add the following ingredients to the sponge.

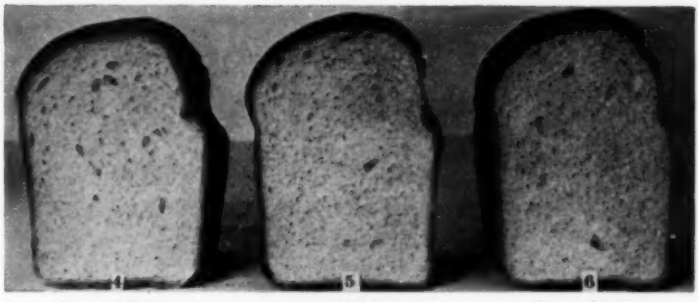
Flour	88 g
Powdered skim milk	9.5 g
Sugar	6.3 g
Fat	6.3 g
Allow to ferment 45 minutes at 27°C.	

A photograph of the bread is shown in Figure 2. It will be noted that Loaf 1, which contained the germ that was simply wetted for only a few seconds, was poor in grain and volume and that with increase in time of steeping up to 5 or 6 hours there was increased improvement in the quality of the loaf. The improvement after 4 hours was, however, only slight. Preliminary experiments had shown that the improvement beyond 7 hours was negligible.

Effect of quantity of germ: Germ steeped for 3 hours was added to the dough to the extent of 2½, 5, 10, 15, and 20%, based upon the weight of the flour. To overcome the effects of proteolytic enzymes, 1 mg of potassium bromate was added to each loaf for each 5% of germ. The control contained no wheat germ and no bromate. Figure 3 is a photograph of the breads. The addition of 2½% of wheat germ improved the quality of the loaf, while 5% seems to have had no deteriorating effects upon the quality of the bread (volume, grain, and texture), as compared to bread containing only white flour. The addition of 15% of germ produced a bread which was very satisfactory from every point of view (see Loaf 5). Even the bread containing 20% of wheat germ could be considered to be of good quality.

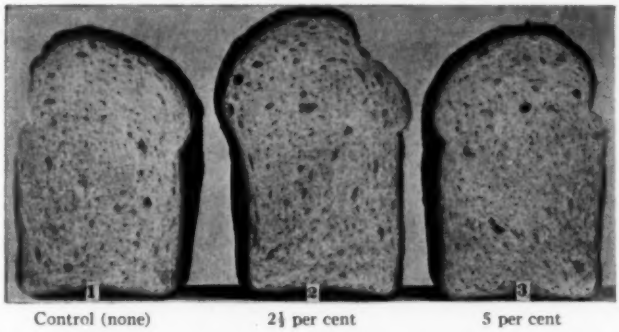


Merely wetted but not steeped | Steeped 1 hr. | Steeped 2 hrs.



Steeped 3 hrs. | Steeped 5 hrs. | Steeped 7 hrs.

Fig. 2. The effect of time of steeping wheat germ on bread-making properties (15% germ).



Control (none) | 2½ per cent | 5 per cent

10 per cent | 15 per cent | 20 per cent

Fig. 3. The effect of quantity of germ on bread-making properties (germ steeped 3 hrs in each case, except the control).

Effect on bread quality of the addition of varying amounts of potassium bromate to doughs containing 20% of germ: Oxidizing agents such as potassium bromate are quite generally used in bread making. Their effects are regarded as due in large part to their counteracting the harmful effects of glutathione. The question arises as to whether addition of potassium bromate, if used in relatively large quantities with dry germ, will produce breads as satisfactory as those produced by the use of steeped germ either alone or with potassium bromate. In the case of breads containing the dry germ (upper row, Fig. 4),

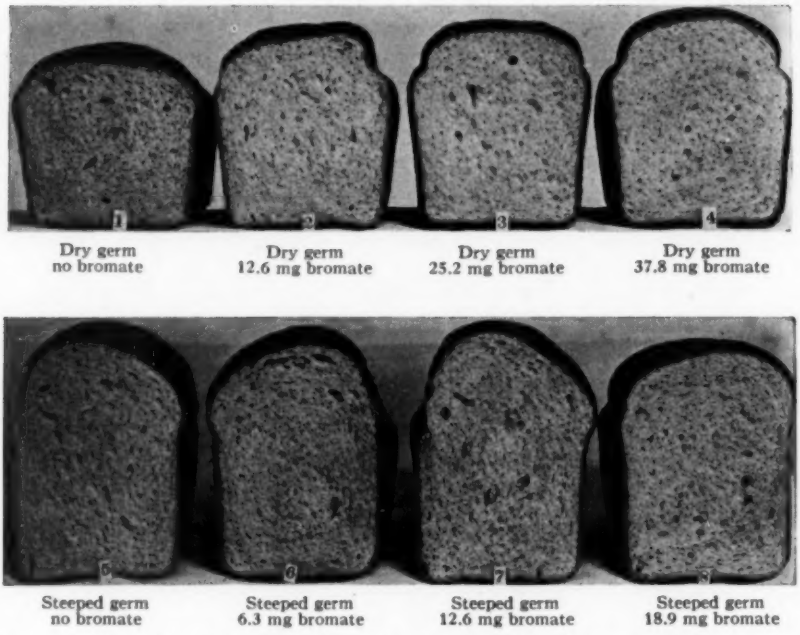


Fig. 4. The effect of varying amounts of potassium bromate with dry and steeped wheat germ used in bread making (one pound loaves) (20% germ).

the baking properties were improved as the amount of bromate was increased. Larger quantities of potassium bromate were not used because 37.8 mg is already in excess of quantities that seem advisable to use in food materials. In the case of doughs containing steeped germ, the bread-making properties were improved as the result of adding up to 12.6 mg of potassium bromate per loaf. Loaf 4, which contained the dry germ and 37.8 mg of potassium bromate, was not as good as Loaf 5, which contained the steeped germ and no bromate. The addition of potassium bromate to dough containing either dry or steeped germ improves its baking properties, but the oxidizing effects

of the bromate do not fully account for all of the improvement in the quality of the bread made with the steeped germ.

Effect of procedure used in making germ bread: Four different baking procedures with essentially the same formula have been developed for making germ bread, and all have proved very satisfactory. Breads made by these four procedures and by two other less satisfactory procedures are shown in Figure 5. In all cases bread was made with 15% of wheat germ and 85% of patent flour. While Loaves 1 and 5 were made by the formula already described, the procedures used in combining the ingredients differed somewhat, as shown in Figure 5.

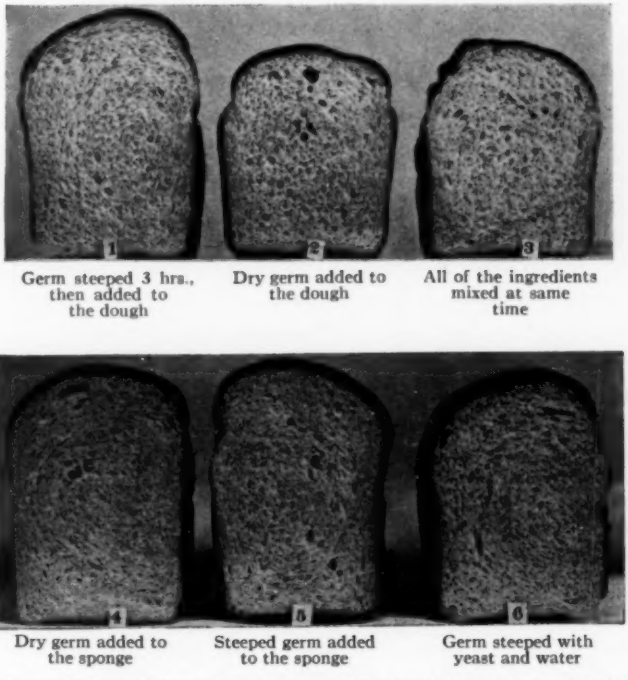


Fig. 5. The effect of the procedure or the manner in which the ingredients are incorporated (15% germ).

Two of the loaves of bread (Nos. 2 and 3, Fig. 5) were of relatively poor quality, while the other four were quite satisfactory. Loaf 2 was a sponge-and-dough bread, the dry germ having been added to the dough. Loaf 3 was made from a straight dough containing dry germ. The other loaves were made by procedures in which the germ had been given some kind of treatment before being added either to the sponge or the dough. In the case of Loaf 1, the germ was steeped 3 hours before being added to the dough; for Loaf 4, the dry germ was added to the sponge 3 hours before the latter was formed into the

dough; and for Loaf 5, steeped germ was added to the sponge. The sponge for Loaf 6, containing nothing but the germ, yeast, and a part of the water, was fermented 2 hours, at the end of which the other ingredients were added to form the dough. A further 2-hour fermentation period was given with a punch at the end of 1½ hours.

Somewhat less yeast (6.3 g) but more sugar (7.8 g) was used for the other five loaves. Water was the only ingredient which in all cases was in contact with the germ in the four procedures which yielded satisfactory bread. Therefore, it is evident that favorable changes take place when germ is in contact with water, whether yeast is present or not. The laboratory work described in this manuscript was completed in May, 1940, and a paper covering the work was read by title at the convention of the American Association of Cereal Chemists that year. Since that time Hullett and Stern (1941) have presented data from which it would appear that yeast is the chief factor in the elimination of the harmful effects of glutathione. The data here reported show that water by itself counteracts the effects of glutathione or other harmful products found in germ.

Germ from different types of wheat: Grewe and LeClerc (1943) made a study of the composition of samples of germ from white winter, soft red winter, hard spring, hard red winter and durum wheat. Baking tests were conducted in which 15% of the flour was replaced by germ from these various types of wheat. This ratio of 85 parts of white flour to 15 of wheat germ was finally agreed upon, because it has been generally admitted that in order to make "germ bread" as rich in thiamin as whole wheat bread, some 15% of germ must be used. Photographs of the bread sections are shown in Figures 6 and 7.

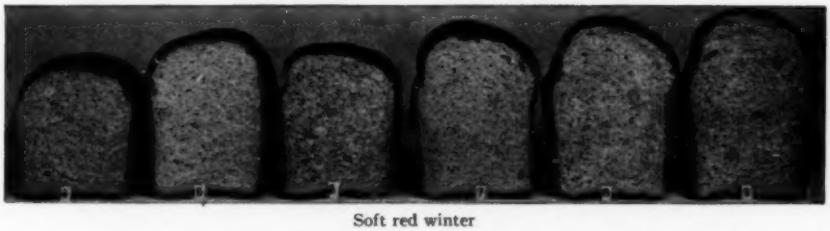
All of the loaves containing germ from soft white winter, soft red winter, hard red winter, and durum wheat were satisfactory. In general, there was a relationship between composition and bread-making properties. Bread made with each of the four samples of germ from hard spring wheat was smaller in volume and poorer in grain and texture than bread in which germ from the other four classes of wheat was used.

Geddes (1930) found that germ added to fifth-middlings flour reduced its baking quality as reflected in poor handling properties of the dough and in the quality of the bread, which had a coarse, open grain and a smaller volume. Since other investigators have encountered similar difficulties, it was quite easy to believe that the addition of wheat germ in the making of bread resulted in marked depreciation in the quality of the loaf.

The two procedures generally used for making bread are a straight dough and a sponge and dough. Many bakers are of the opinion that

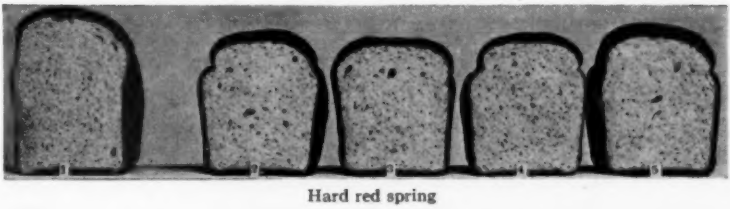
flour substitutes, adjuncts, or improvers should be added at the dough stage rather than to the sponge. Our experience indicates that wheat germ should preferably be added to the sponge or should be given a preliminary steeping in water. When treated in either of these two ways very good wheat germ bread can be made, as we have shown here.

Soft white winter

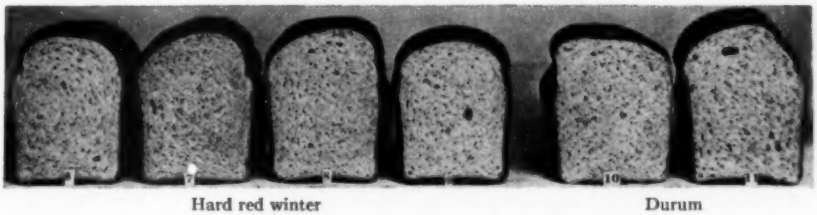


Soft red winter

Fig. 6. Bread made with 15% wheat germ and 85% patent flour.



Hard red spring



Hard red winter Durum

Fig. 7. Bread made with 15% wheat germ and 85% spring patent flour.

The effect of adding sodium chloride to wheat germ: The addition of salt to the germ (in quantities normally used in baking bread) has a very marked effect on the ease with which the dough is handled. Our experiments show that when salt and water are added to the germ

the colloidal properties of the germ are changed, a jell-like mass being formed.

Five mixtures of 10 g of germ with 50 ml of water containing 0, 1, 2, 3 and 4% of sodium chloride were prepared and transferred to glass cylinders. Ninety minutes later the photograph shown in Figure 8

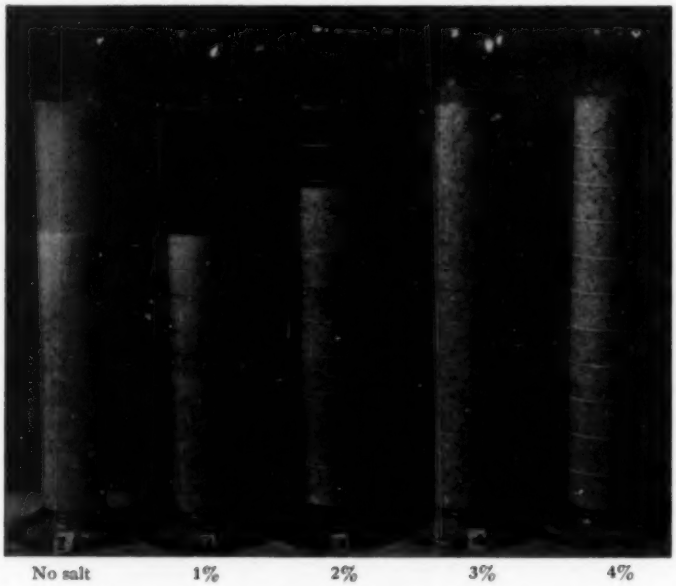


Fig. 8. Effect of variation in the strength of sodium chloride solution on apparent viscosity of wheat germ (30 g germ to 150 ml solution).

was taken. It will be noted that there was a decrease in the depth of liquid above the suspension of germ with increase in the concentration of sodium chloride. The liquid which separated from the mixture containing no salt was milky in appearance, while that from mixtures containing salt was clear. Cylinder 5 in Figure 6 contained salt in the proportion most nearly approximating that used in bread making.

The viscosities of similar preparations were determined by use of a MacMichael viscometer. The results as charted in Figure 9 show that the addition of sodium chloride caused a very marked increase in viscosity. For example, the germ mixture in which no salt was used had a viscosity of 20° MacMichael, while that containing 3% of salt had a viscosity of 680° MacMichael.

Diastatic power: The improvement in baking properties observed when wheat germ is steeped before addition to flour dough very probably results from a number of factors, rather than just one. Since enzyme activity is known to be important in bread making, a study

was made of the quantity of maltose produced by the addition of steeped germ.

It is well known that steeping of wheat and barley results in increased amylase activity. It was noted that with an increase in time of steeping of wheat germ added to flour dough, there was an increase

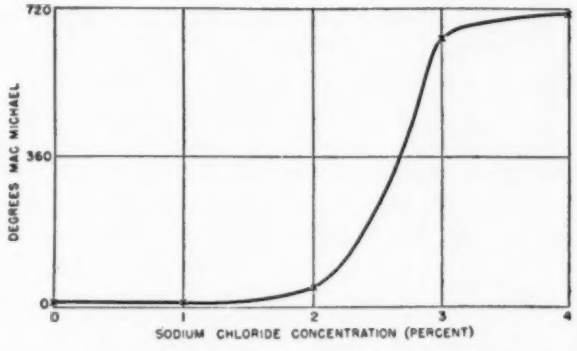


Fig. 9. Effect of variation in concentration of sodium chloride solution on apparent viscosity of wheat germ (30 g germ to 150 ml solution).

in the intensity of the color of bread crust, indicating an increase in the amount of sugars present. This suggested that there was an increase in the amylase activity of wheat germ produced as a result of steeping. Diastatic power determinations were made on extracts prepared by steeping germ for varying lengths of time. The procedure used was a modification of the method for the determination of the diastatic power of malt as adopted by the Association of Official Agricultural Chemists (1935). The results showed that with increase in time of steeping, during the first 7 to 8 hours, there was a sharp increase in diastatic power (Fig. 10).

Oxidation-reduction: Twenty ml of extract, equivalent to 4 g of the germ, was placed in a 250-ml Erlenmeyer flask; 4 ml of 12*N* H_2SO_4 was added; a known excessive amount of 0.01*N* iodine solution was added, followed by 3 ml of a 0.5% starch solution. The extract-mixture was then titrated with standard $Na_2S_2O_3$ solution to the disappearance of the characteristic blue color. The total volume of the iodine solution used in the titration minus the iodine equivalent of the $Na_2S_2O_3$ solution represented the iodine necessary to oxidize the reducing agents present in the extract-mixture. Data showing the amount of iodine consumed per gram of germ steeped for varying lengths of time are given in Figure 10. The extract from 1 g of germ which had been in contact with water for 30 minutes required 1.06 ml of the standard iodine solution for oxidation. The extract of germ steeped 24 hours required only 0.64 ml of iodine. The oxidation of

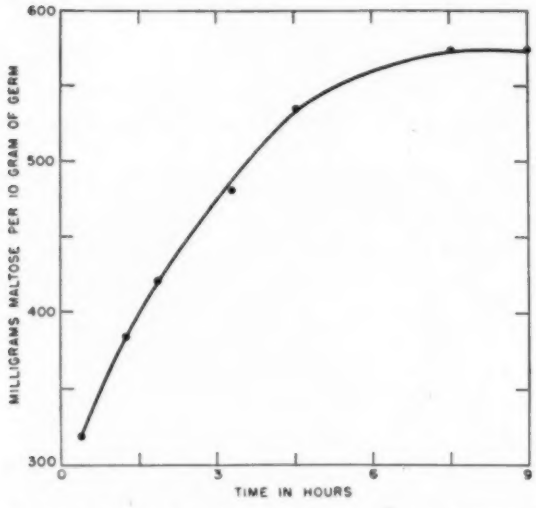


Fig. 10. Effect of time of steeping germ on its production of maltose (10°C).

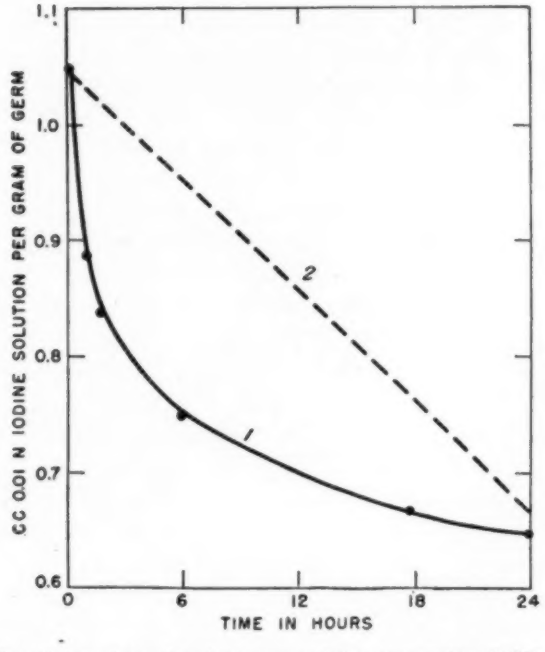


Fig. 11. Effect of time of steeping wheat germ on iodine consumption (10°C). Curve 1 = Extract separated after steeping for various periods of time. Curve 2 = Extract separated 30 minutes after steeping and tested immediately, and again after 24 hours.

the germ extract obtained during the first 6 hours was the most rapid.

Tests were also conducted to determine whether the substances causing oxidation were soluble in water. This was done by saving a portion of the extract which had been in contact with the germ for 30 minutes and testing it again at the end of 24 hours, at which time it required only 0.66 ml of 0.01*N* iodine for oxidation. This is regarded as evidence that the substances causing oxidation are soluble in water and retain that property when separated from the residue. Tests were made on three samples of germ. Curves similar to those shown in Figure 11 were obtained in all cases.

From this experiment it is evident that oxidation took place during the time the wheat germ was in contact with water. Von Kuthy (1937) showed that ascorbic acid increased during germination. Melville and Shattock (1938) found that flour contains an ascorbic acid oxidase. Sullivan, Howe, and Schmalz (1936) isolated glutathione from wheat germ. Hopkins and Morgan (1936) found that when ascorbic acid and glutathione are together in the presence of the ascorbic acid oxidase, the glutathione wholly protects the ascorbic acid from oxidation, while the glutathione itself is oxidized at a rate which is exactly the same as that at which ascorbic acid is oxidized when alone. These facts indicate that in our water extract of wheat germ, glutathione was being oxidized by the dehydroascorbic acid produced by the oxidase, or perhaps by the oxidase itself.

Doughs made with the dry germ have a sticky property which cereal chemists regard as due to proteolysis. Steeped germ, when incorporated in the mix, does not impart this characteristic sticky property to the dough.

A change in oxygen consumption indicates a change in the balance of two effects, one due to reducing agents such as glutathione and ascorbic acid and the other to oxidizing action of the oxidases. Oxidation-reduction potentials might have been of value in interpreting the data, but this phase of the work was beyond the planned scope of this investigation.

Summary

The term "steeping" as used in this paper applies to the process in which water is added to wheat germ and the two allowed to stand for a period of time, usually from 1 to 7 hours.

The steeping of wheat germ causes a marked improvement in its bread-making properties when added to flour dough. The extract of momentarily wetted germ, when separated and incorporated in bread dough, produces somewhat inferior loaves, whereas the extract of steeped germ produces a loaf as good as, or even better than, the control (Fig. 1).

The beneficial effect of steeping increases with increase in time up to 6 or 8 hours. The addition of potassium bromate, along with germ, results in an improvement in bread-making properties.

Steeped germ up to 10% can be added to flour dough without appreciable detrimental effects on the quality of the loaf. The addition of 2½% or 5% of steeped germ may give even better bread than when no germ is used. The use of 15 to 20% produces a very satisfactory bread.

In a study on germ from different classes of wheat very satisfactory bread was produced when germ from white, soft red winter, hard red winter, and durum wheats was used. Germ from hard spring wheat was not so satisfactory for bread as was germ from the other classes of wheat.

The addition of salt (in amounts normally used in bread making) to the germ during steeping causes an improvement in the handling properties of the dough. The colloidal properties of the germ as measured by viscosity are appreciably changed by the addition of salt solutions.

The steeping of wheat germ results in an increase in its diastatic power. There is also a decrease in oxidizable substances. These changes are quite rapid at first, with some change taking place over a period of 7 hours.

In order to produce the best bread, the germ should be steeped for about 3 hours before being added to the dough or to the sponge.

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THE EFFECT OF MATURITY UPON THE QUALITY OF HARD RED SPRING AND DURUM WHEATS [1]

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North Dakota Agricultural Experiment Station, Fargo, North Dakota

(Read at the Annual Meeting, May 1942)

The investigation reported here was undertaken to obtain information regarding the effect of maturity on certain properties of hard red spring and durum wheats. The results may be of value in the interpretation of information obtained in pre-harvest wheat surveys for the purpose of forecasting wheat quality. Advantage likewise may accrue to the grain producer who can commence harvest at a relatively early date, with consequent saving of labor, provided no significant sacrifice in quality is entailed. Early harvest is also important in the Great Plains area when grasshopper infestations threaten to reduce yield.

A number of papers may be found in the literature relative to the effect of maturity on wheat quality. Humphries and Biffen (1907) found no significant differences in baking strength in wheat cut at green, ripe, and dead ripe stages, and Stoa (1924) found only minor variations in protein content and baking quality of early- and late-harvested Marquis wheat. Very similar conclusions were reached by Mangels and Stoa (1928), though there were slight differences in baking quality in favor of the riper samples. Wilson and Raleigh (1929) found that loaf volumes of Marquis wheat flours were larger for the immature samples, but color and texture improved with degree of ripeness.

Sharp (1925) thought that the density of wheat was not greatly affected by the stage of maturity at which the wheat was harvested, although Mangels and Stoa (1928) showed that immature Marquis had a lower test weight than mature wheat. Sharp and Elmer (1924) found evidence that slight changes in the protein fractions took place as the kernel developed, without much change in the quantity of total

[1] The investigations reported in this paper were principally supported by funds contributed through Bankhead-Jones Project No. 18–1207 "Research in the Statistics of Agriculture and the Associated Statistical Theory," in which the Bureau of Plant Industry and the Agricultural Marketing Service of the Department of Agriculture cooperated with the Experiment Station.

protein. Sharp (1925) reported a decrease in the percentage of amino acid with kernel development.

Newton and McCalla (1934, 1935) and McCalla and Newton (1935), in a series of extensive studies on the effect of wheat maturity on physical properties, chemical composition, and baking strength, found that 58% dry matter content represented a critical stage in the development of Canadian hard red spring wheat. Samples harvested after this point were approximately mature.

Miller (1939) presented detailed data on changes in composition of winter wheat at different stages of development. The nitrogen content of the grain increased from the commencement of formation until maturity.

Plan of Experiment

An experiment was laid out in which three varieties of hard red spring wheat—Thatcher, Ceres, and Premier—were planted in replicated plots at Fargo. In addition, one variety of durum was also planted. Two dates of planting were used. The first, denoted as "early" in this investigation, coincided closely with the normal seeding, while the second or "late" series was distinctly late in respect to common practice. The wheats were planted in a randomized block arrangement, four replications of each wheat being used for each date of planting. Early and late seedings were included in each block. Individual plots were 210 feet long and 6 feet wide with 18-inch alleys between plots.

For sampling, the plots were divided in the center and samples taken at random from each half plot. A 10-foot area at the end of each plot was left as a guard and not sampled. Sample areas were chosen at random in the laboratory prior to sampling by the use of Tippett's random sampling numbers and the areas so found were located in the plots by measurement from the ends of each half plot. Individual sample units were 1×4 feet in size, taken by placing a rectangular wooden frame across the plot to include 8 drill rows. All plants within the quadrat frame were pulled and reserved for detailed examination. These units were taken from the center of the plot, leaving two outside rows at either end of the quadrat frame unsampled. A 1-foot strip was also left between adjacent sample units. For the pre-harvest samples 3 units were taken from each half plot making a total of 6 for each plot or 24 composited for any one variety. At maturity 4 units were taken from each half plot, making a total of 32 individual samples composited for each variety.

The first samples were taken 12 days before what was considered to be the normal harvest date. The normal date was estimated by adding 30 days to the date on which head emergence took place. This

period is rather longer than the average but approached quite closely the normal period for the 1941 season at Fargo. The samples were taken at 2-day intervals from the first sampling until the 30-day period had elapsed. The heads were immediately removed from the straw, and air-dried under laboratory conditions. The heads were then run through a miniature thresher, and the hulls and similar material removed by winnowing. Figure 1 shows the apparatus used

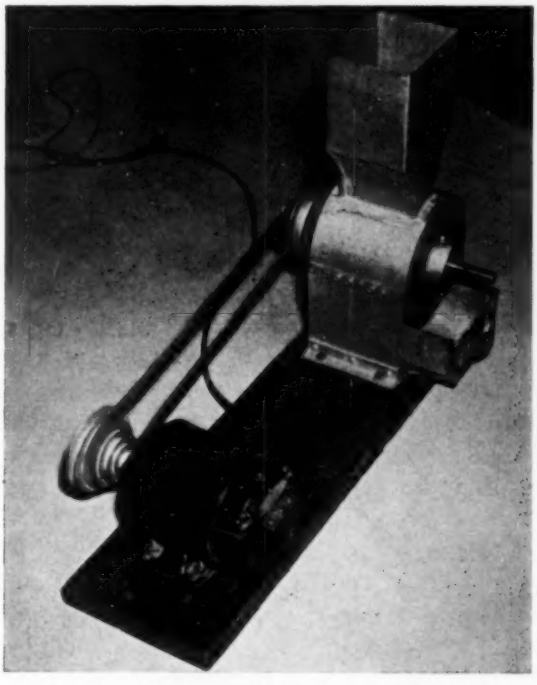

Fig. 1. Apparatus used to thresh head samples from individual sample units.

in threshing the samples. Following threshing the samples were stored in paper sacks in a dry room for several weeks prior to milling. The average moisture content of the samples was 10.7%. It is realized that the composition and possibly some of the properties of the more immature samples would be changed slightly from corresponding values at the time the grain was actually cut. McCalla and Newton (1935) have shown that loss of carbon occurs owing to respiratory activities during air drying of immature material, but that this method of drying more nearly approximates field conditions of curing than does more rapid drying. There is also the probability that more drastic drying would alter the baking quality.

The samples, which were too small for milling with the regular Allis-Chalmers experimental mill, were cleaned and scoured. Aliquots were taken for moisture and protein determinations. Test weight and grade were also determined, the former by a micro method described by Harris and Sibbitt (1942). When sufficient wheat was available 175 g was used for milling; otherwise 100 g was employed. These millings were done on a micro mill with the production of a long-patent or straight-grade flour by essentially the method developed by Geddes and Aitken (1935) and employed by Harris and Sanderson (1939). These flours were then baked by a micro formula, employing 25 g of flour, 0.3% high diastatic malt, 0.001% $KBrO_3$, 0.1% ammonium phosphate (monobasic), 2.5% sucrose, 1.0% NaCl, and 3% yeast. A 3-hour fermentation period was used. The doughs were mixed for 2½ minutes in a Hobart mixer equipped with special dough hooks. Micro-baking methods have been described by Geddes and Aitken (1935), Harris and Sanderson (1938), Van Scoyk (1937, 1939), and others.

In addition to the samples taken at 2-day intervals, four 10-pound samples of Thatcher and Mindum were collected at different stages of maturity from the same field experiment in order to have a sufficient quantity of material for further studies regarding the effect of maturity upon biochemical properties of the gluten and on macaroni quality. The Thatcher series was milled on the Allis-Chalmers experimental mill, while the Mindum was milled and the resultant semolina processed by techniques described by Harris and Sibbitt (1942). Glutens were washed from these larger samples and their dispersibilities in 10% sodium salicylate solution were determined. The increase in viscosity with time of dispersion was ascertained by means of the Ostwald capillary pipette according to a procedure described by Harris and Johnson (1941). Changes in protein concentration in the liquid were also found. Analytical methods conformed to those described in *Cereal Laboratory Methods* (4th ed.).[2]

Mixogram curves were obtained on the flour samples milled from the Thatcher wheat, and these flours were also baked by the malt-phosphate-bromate method with 3-hour fermentation and 100 g of flour.

Discussion of Results

Descriptive data with wheat protein and milling yields are shown in Table I, while the baking data are shown in Table II. In the case of the late plantings the 8- and 6-day samples of Ceres were not obtained and the values at these dates therefore represent the averages

[2] Published by American Association of Cereal Chemists, 110 Experiment Station Hall, Lincoln, Nebraska.

TABLE I

MILLING AND VARIOUS OTHER DATA COLLECTED ON THE HARD RED SPRING WHEAT SAMPLES HARVESTED AT DIFFERENT STAGES OF MATURITY

Variety	Days before final cutting	Maturity of grain	Protein [1]	Vitreous kernels	Test weight	Grade [2]	Flour yield	Ash [1]
			%	%	*lbs/bu*		%	%
EARLY PLANTING								
Thatcher	12	Green	13.5	40	52.1	5 NS	64.5	0.59
"	10	Green	13.2	65	53.8	4 NS	65.7	0.58
"	8	Green	14.0	75	55.8	3 DNS	65.6	0.56
"	6	Sl green	14.2	85	55.9	3 DNS	65.0	0.56
"	4	Ripe	14.2	95	55.8	3 DNS	65.8	0.55
"	2	Ripe	14.2	95	55.6	3 DNS	67.3	0.61
"	Harvest	Ripe	14.7	95	55.5	3 DNS	66.9	0.58
Premier	12	Green	13.4	65	54.0	4 NS	66.8	0.68
"	10	Green	13.6	70	57.8	2 NS	68.6	0.65
"	8	Green	14.2	85	58.6	1 DNS	67.3	0.63
"	6	Sl green	14.4	85	58.7	1 DNS	69.8	0.63
"	4	Sl green	15.0	90	59.0	1 DNS	70.0	0.60
"	2	Ripe	14.9	95	58.6	1 DNS	70.5	0.68
"	Harvest	Ripe, Br Kern	15.1	95	58.1	1 DNS	71.0	0.63
Ceres	12	Green	12.7	65	52.0	5 NS	61.1	0.58
"	10	Green	13.4	75	53.5	4 DNS	62.3	0.59
"	8	Green	13.4	85	52.7	5 DNS	64.1	0.63
"	6	Sl green	13.9	90	54.2	4 DNS	66.4	0.61
"	4	Ripe	14.0	95	54.3	4 DNS	64.6	0.54
"	2	Ripe	14.1	95	53.8	4 DNS	63.4	0.56
"	Harvest	Ripe	14.0	95	52.7	5 DNS	65.6	0.56
LATE PLANTING								
Thatcher	8	Green	15.9	70	55.6	3 NS	69.7	0.70
"	6	Green	16.2	75	55.2	3 DNS	65.3	0.69
"	4	Sl green	16.0	80	55.0	3 DNS	71.8	0.73
"	2	Sl green	15.8	95	55.0	3 DNS	68.6	0.65
"	Harvest	Sl green	16.1	95	55.0	3 DNS	63.7	0.61
Premier	8	Green	15.3	70	56.7	3 NS	66.8	0.74
"	6	Green	14.6	80	55.0	3 DNS	67.5	0.71
"	4	Sl green	15.2	85	57.5	2 DNS	67.0	0.71
"	2	Sl green	15.4	90	57.3	2 DNS	67.7	0.67
"	Harvest	Sl green	15.6	95	55.0	3 DNS	65.4	0.66
Ceres	4	Sl green	15.0	75	52.7	5 DNS	67.9	0.73
"	2	Sl green	15.3	90	52.9	5 DNS	67.3	0.76
"	Harvest	Sl green	14.8	95	53.7	4 DNS	62.2	0.61
10-POUND SAMPLES MILLED ON ALLIS-CHALMERS EXPERIMENTAL MILL								
	Harvest date							
Thatcher	July 19	—	13.2	20	39.9	SGRS	39.8	0.76
"	July 24	—	13.1	60	51.7	5 NS	59.0	0.54
"	July 28	—	13.9	75	57.2	2 DNS	66.7	0.44
"	Sept. 3	—	14.8	95	54.4	4 DNS	67.7	0.39

[1] Results calculated to 13.5% moisture basis.
[2] Letters denote first letter of each word in assigned grade; *e.g.*, DNS = Dark Northern Spring.

TABLE II

BAKING DATA ON THE FLOURS MILLED FROM HARD RED SPRING WHEAT SAMPLES HARVESTED AT DIFFERENT STAGES OF MATURITY

Variety	Days before final cutting	Absorption [1]	Loaf volume	Crumb grain [2]	Crumb color [3]	Symmetry [4]
		%	cc			
EARLY PLANTING						
Thatcher	12	64.6	157	5.8 O	4.2 y	4.5 o
"	10	64.6	162	6.5 O	4.8 y	4.5 o
"	8	64.6	164	6.2 O	5.0 y	4.5 o
"	6	63.6	174	7.0	5.0 y	4.5 o
"	4	63.6	179	7.0	6.0 y	4.5 o
"	2	62.6	188	7.2	6.0 y	4.5 o
"	Harvest	62.6	176	7.2	6.8 y	4.5 o
Premier	12	68.6	164	5.8 C, O	6.0 y	4.5 o
"	10	68.6	158	6.5 O	6.5 y	4.5 o
"	8	67.6	162	6.0 C, O	6.5 y	4.5 o
"	6	67.6	168	6.2 O	7.0	4.5 o
"	4	66.6	158	6.2 O	7.0	4.5 o
"	2	66.6	149	6.2 O	5.0 g-y	3.8 o
"	Harvest	66.6	127	6.0 C	4.5 g-y	2.2 o
Ceres	12	67.6	143	6.5 O	5.0 y	3.5 o
"	10	67.6	158	6.5 O	5.0 y	4.2 o
"	8	65.6	171	6.5 O	6.0 y	4.5 o
"	6	64.6	161	6.8 O	6.0 y	4.5 o
"	4	64.6	159	6.5 O	6.2 y	4.5 o
"	2	64.6	163	6.5 O	6.5 y	4.5 o
"	Harvest	64.6	151	6.5 O	5.5 y	3.0 o
LATE PLANTING						
Thatcher	8	65.6	197	6.5 O	7.5	4.5 o
"	6	63.6	197	6.8 O	7.0	4.5 o
"	4	63.6	195	6.5 O	7.0	4.5 o
"	2	62.6	193	6.5 O	6.5	4.5 o
"	Harvest	61.6	166	6.5 O	6.5	3.8 o
Premier	8	67.6	166	6.5 O	6.5	3.8 o
"	6	66.6	161	6.8 O	6.5	3.5 o
"	4	65.6	141	6.0 O	6.5	3.5 o
"	2	64.6	131	6.2 O	6.5	3.0 o
"	Harvest	63.6	139	6.0 O	6.5	3.0 o
Ceres	4	66.6	173	6.5 O	6.0 g-y	3.8 o
"	2	66.6	173	6.5 O	6.0 g-y	3.5 o
"	Harvest	65.6	169	6.5 O	7.0 y	4.0 o
10-POUND SAMPLES OF THATCHER—BAKED BY 100-G METHOD						
	Harvest date					
Thatcher	July 19	63.4	620	5.0 C, O	2.0 g-y	3.0 o
"	July 24	61.7	675	6.5 O	4.8 g-y	4.5 o
"	July 28	58.7	715	7.8	5.5 g-y	4.5 o
"	Sept. 3	57.3	660	7.5	6.5 y	4.0 o

[1] Results calculated to 13.5% moisture basis.
[2] Grain: O = open, C = coarse, perfect score = 10.
[3] Color: y = yellow, g-y = gray-yellow, perfect score = 10.
[4] Symmetry: o = overoxidized, perfect score = 5.

for Thatcher and Premier only. This method of combining the data from three varieties into a single value is open to objections, inasmuch as individual varietal differences are smoothed out, but a result typical of the trends to be expected from the hard red spring wheat crop in

TABLE III

RELATION OF SOME RECORDING MICRO DOUGH MIXER CURVE PROPERTIES TO KERNEL AGE

Variety	Harvest date	Absorption [1]	Height at maximum	Time at maximum height	Height after 10 minutes' mixing
		%	*cm.*	*min.*	*cm.*
Thatcher	July 19	63.4	8.2	.9–1.8	6.9
"	July 24	61.7	8.3	3.6–4.5	7.5
"	July 28	58.7	8.7	3.6–4.5	7.5
"	Sept. 3	57.3	9.1	4.5–5.4	7.7

[1] Results calculated to 13.5% moisture basis.

general should be represented by this procedure. Mixogram data obtained on four samples of Thatcher are shown in Table III. The maximum height of the curve, as well as mixing time, increased consistently with kernel age.

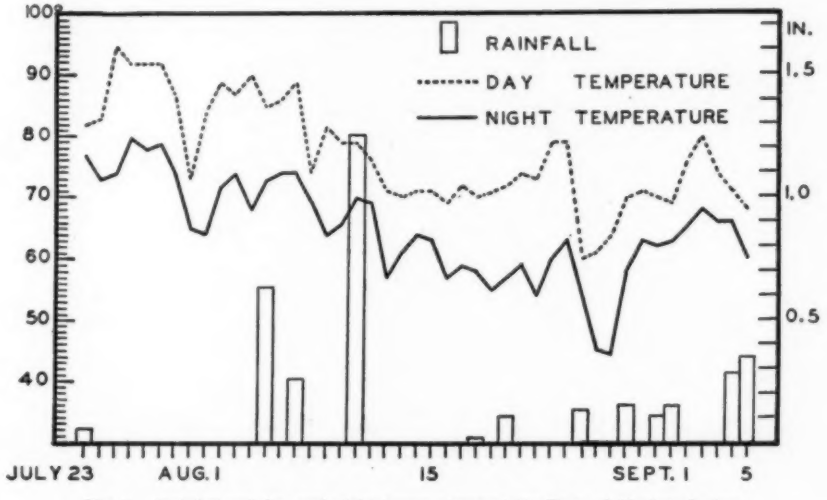

Fig. 2. Rainfall and day and night temperatures at the Fargo field plots during the wheat-ripening and post-harvest period.

Figure 2 represents the rainfall and day and night temperature ranges which occurred during the harvest period. It will be noticed that there was a heavy precipitation on August 11, followed by cool weather approximately two days before the late-planted series was ready to cut.

Figure 3 shows the effect of maturity upon the percentage of vitreous kernels, test weight per bushel, and total flour yield for the two series of wheats. The influence of cutting date upon vitreous

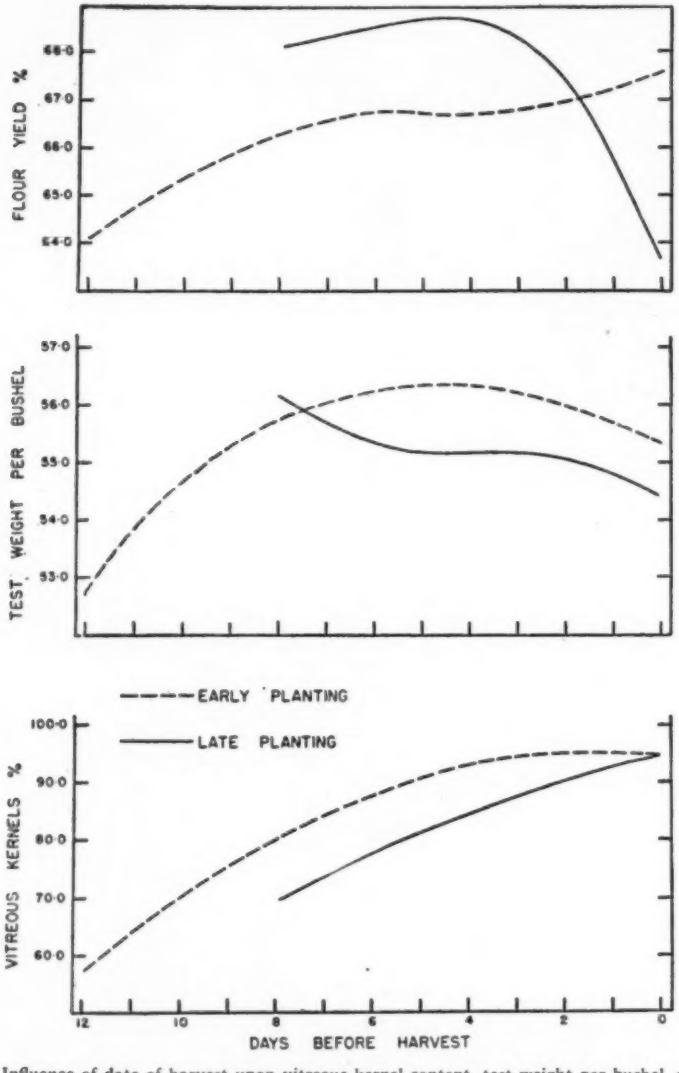

Fig. 3. Influence of date of harvest upon vitreous kernel content, test weight per bushel, and flour yield of three varieties of hard red spring wheat planted at two different times.

kernel content is quite marked for both series, vitreousness increasing to a maximum as the grain ripens. The values for the early-planted wheats are higher than for those late-planted up to the date of final cutting. The test weight results show a somewhat similar trend in

the early plantings, but have a tendency to decline after the maximum was apparently reached four days before harvest. The late-seeded wheats, however, appear to decrease in test weight as ripening

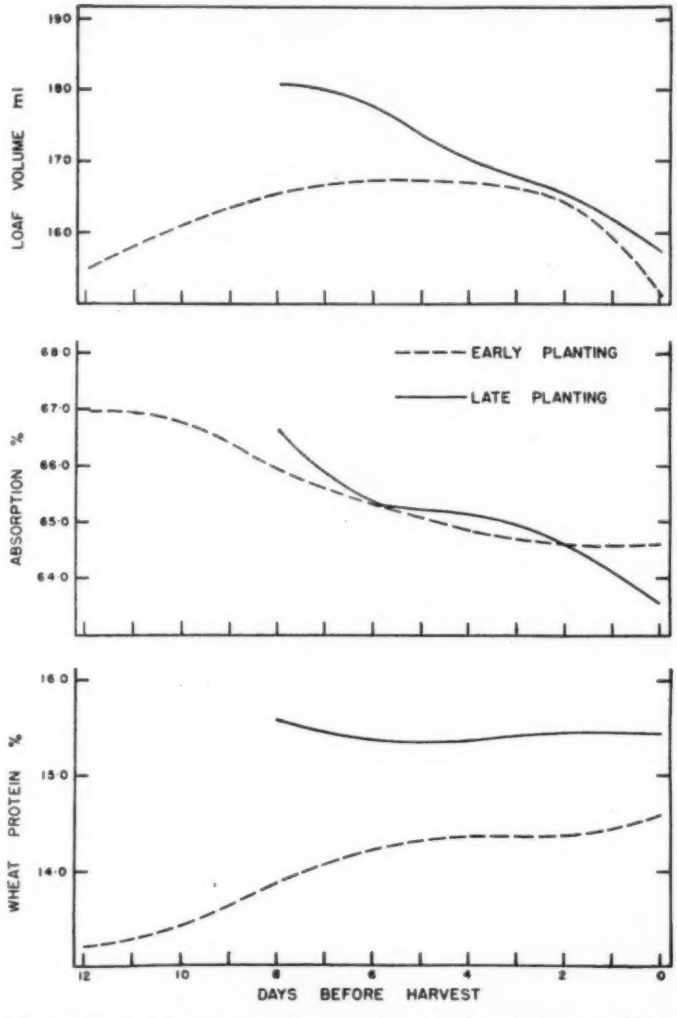

Fig. 4. Influence of date of harvest upon wheat protein content, flour absorption, and loaf volume of three varieties of hard red spring wheat planted at two different times.

progresses. Flour yields in general paralleled the test-weight values, except for the more mature samples in the late series.

Figure 4 shows the trends between harvest date and wheat protein content, flour absorption, and loaf volume. The wheat protein content increased in the early-planted wheats as maturity approached. No

significant differences are noticeable in the late-planted samples, but all these values are higher than in the first series. The absorption of the flours decreased as ripening progressed in both the early and late series; the same effect was also noticed in the Mindum when the macaroni was processed. The loaf volume increased to a maximum

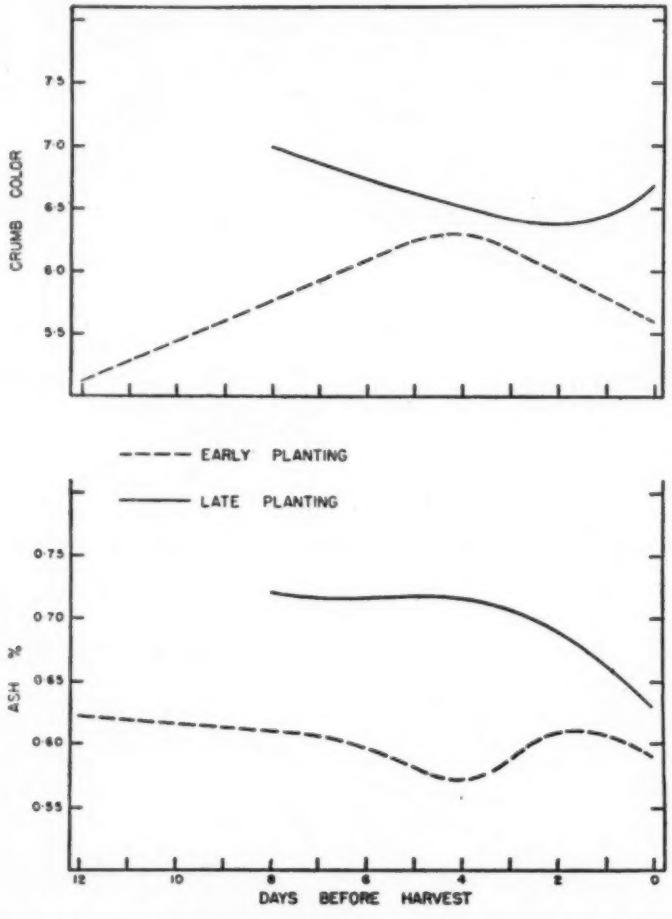

Fig. 5. Influence of date of harvest upon flour ash and crumb color of three varieties of hard red spring wheat planted at two different times.

shortly before maturity in the early planting, then decreased sharply for the final sample, while the late wheats showed a consistent decline from 8 days before normal harvest to the final cutting. The cause for these unexpected changes in loaf volume is not apparent at the present time.

Figure 5 shows the ash contents of the flours and the crumb colors of the loaves baked therefrom in relation to date of harvest. Ash content had a tendency to decrease as the ripening progressed, especially in the late-planted series. This trend reflects the increased ease of milling the more mature samples. No reason is evident for the decrease in ash 4 days before normal harvest in the early-planted series, followed by a later rise. Crumb color showed a distinct maximum at this point and then fell with increasing ripeness. In the late-planted series, color decreased slightly until 4 days before harvest, and then increased to the final value at the initial cutting.

Little harmful effect upon bread wheat quality from harvesting wheat slightly before normal ripeness was demonstrated by this study, and in the late-planted series it appeared to have a favorable influence. Late-seeded wheats were higher in protein content and flour yield, if cut early, than the late series, but produced flours of higher ash content.

Descriptive, analytical, and milling data from four samples of Mindum durum harvested at different dates are presented in Table IV.

TABLE IV

MILLING AND OTHER DATA OBTAINED ON FOUR SAMPLES OF MINDUM HARVESTED AT DIFFERENT STAGES OF MATURITY

Harvest date	Maturity of grain	Protein [1]	Vitreous kernels	Test weight	Grade [2]	Semolina yield	Ash [1]
		%	%	*lbs/bu*		%	%
July 28	Green	14.2	60	52.7	5 AD	37.3	0.71
July 31	Sl green	14.3	70	56.1	3 AD	42.8	0.70
Aug. 4	Sl green	14.7	80	58.3	2 HAD	43.2	0.70
Sept. 3	Bleached & sl green	15.4	85	58.2	2 HAD	44.1	0.65

[1] Results calculated to 13.5% moisture basis.
[2] Letters denote first letter of each word in assigned grade, *e.g.*, HAD = Hard Amber Durum.

The normal harvest date would be approximately 2 days following August 4, while the final date, September 3, represents a very late post-harvest sample. No substantial amount of rain had fallen between the date at which this sample was taken and August 4. The sample had bleached slightly, however. The wheat protein, vitreous kernel content, and semolina yield increased with maturity. The post-harvest sample produced the lowest ash.

The macaroni processing data for the four samples of semolina are seen in Table V. The semolina protein content increased with maturity of the wheat, while the number of specks remained constant with the exception of the sample nearest the normal harvest date, which contained approximately twice the number present in the other samples. The absorption decreased steadily with maturity, while the

macaroni color score increased in the same order. The effect of immaturity upon macaroni color was very evident, and emphasized the importance of the degree of ripeness before cutting durum wheat. Immature wheat apparently produces semolina requiring more water to form a dough of optimum consistency for macaroni manufacture. The results of too early cutting upon quality are apparently more marked in the instance of durum as compared with bread wheat.

TABLE V

MACARONI PROCESSING DATA ON THE FOUR SAMPLES OF DURUM SEMOLINA

Harvest date	Protein [1]	Specks per 10 sq in semolina	Absorption [1]	Macaroni color
	%		%	*Perfect score 10*
July 28	14.1	10	28.9	2.0 deep brown
July 31	13.8	10	27.6	5.0 sl brown
Aug. 4	14.7	20	22.4	6.0 deep yellow & sl brown
Sept. 3	14.8	12	22.2	8.0 good translucent yellow

[1] Results calculated to 13.5% moisture basis.

Glutens were washed from the eight large samples of Thatcher and Mindum after milling, by methods described by Harris (1938) and 10 g of the gluten was then dispersed in 100 ml of 10% sodium salicylate solution on a rotary shaker. Measurements of viscosity were made every 2 hours for 24 hours by means of the Ostwald capillary pipette, while the concentration of dispersed protein was likewise determined. The data are shown in the form of graphs of viscosity and concentration against dispersion time in Figures 6 and 7. The curves in Figure 6 tend to form two groups corresponding to the class of wheat from which the glutens were prepared. The viscosities of the four Mindum glutens increased more rapidly than the Thatcher and came to equilibrium earlier, with the possible exception of the first Thatcher. The order of viscosity increase with time for the Mindum glutens corresponded with the order of harvest. In the Thatcher gluten dispersions, on the other hand, the increase in viscosity was in the opposite order.

In both wheats gluten dispersibility decreased with maturity. To determine whether consistent differences existed between the rates of increase in viscosity and concentration of proteins in the dispersions, the tangent of the angle made by a line drawn tangent from the curves at the eighth hour to the base line was calculated. This particular point of the dispersion period was chosen because maximum differences in the slopes of the different curves become most marked at this time. Harris, Olson, and Johnson (1942) have shown that at later periods the rates change, and in some instances decrease greatly. It was also

found that viscosity in 10% sodium salicylate dispersions after 6 and 8 hours of dispersion are very closely related. The lengths of the lines forming the tangent were measured and their ratios found. The differences between the values of the tangents, as determined from the viscosity curves and the corresponding tangents of the concentration curves, were also found. Table VI shows the data as computed with

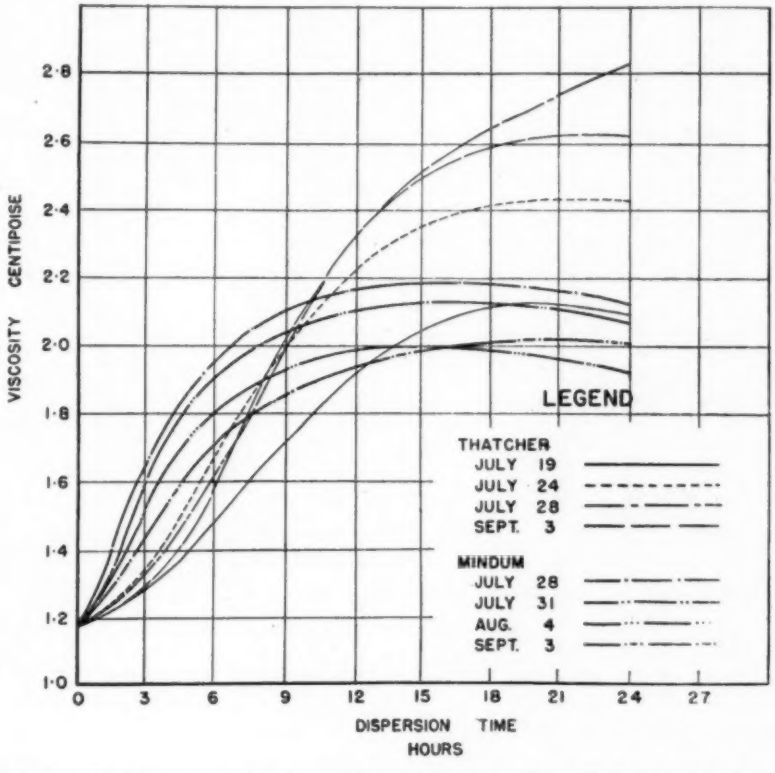

Fig. 6. Relationships between viscosity and dispersion time in sodium salicylate solution of glutens prepared from Thatcher and Mindum wheats harvested at different dates.

the numerical values of the tangents. The differences between the tangents for the viscosity and concentration curves are also shown. It will be noticed that this difference became increasingly negative with wheat maturity in the instance of the Mindum samples, while in the Thatcher dispersions the difference increased positively with length of time after heading.

These results would seem to indicate a change in the constitution of the gluten-protein complex during the ripening period. From previous work published (Harris and Johnson, 1940), it has been shown that hard red spring wheat gluten dispersions in sodium salicylate have

relatively higher viscosities than durum wheat. It was accordingly postulated that hard red spring wheat gluten dispersions have larger protein micelles than durum wheat gluten dispersions. In the present study, the differences in the slope of the lines from the viscosity and concentration curves become increasingly positive in the case of Thatcher, while for Mindum this difference became more negative with

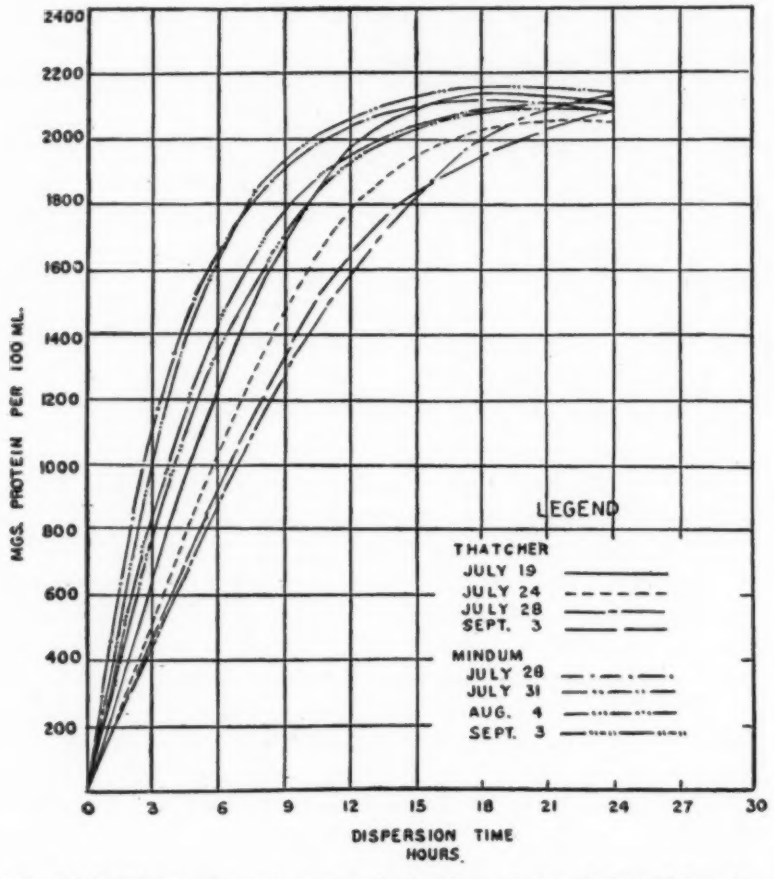

Fig. 7. Relationship between protein concentration in sodium salicylate solution and dispersion time of glutens prepared from Thatcher and Mindum wheats harvested at different dates.

lapse of time after heading. This apparently suggests that the Thatcher gluten micelles became larger as ripening progressed, while for Mindum the opposite was true and the micelles decreased in size. In other words, the characteristic difference between Thatcher and Mindum gluten particles increased steadily with time after heading. One might theorize from these trends that the sizes of the gluten

TABLE VI

TANGENTS OF ANGLES COMPUTED FROM THE VISCOSITY AND CONCENTRATION DATA

Variety	Date of harvest	Viscosity data—tangent	Concentration data—tangent	Differences between the tangents of viscosity and concentration curves
Thatcher	July 19	1.4000	1.9750	−0.5750
"	July 24	2.1000	1.3083	0.7917
"	July 28	2.2439	1.1607	1.0832
"	Sept. 3	2.6571	1.1574	1.4997
Mindum	July 28	2.7875	2.9714	−0.1839
"	July 31	2.1400	2.4054	−0.2654
"	Aug. 4	1.5102	1.8864	−0.3762
"	Sept. 3	1.3043	1.7273	−0.4230

micelles may be nearly the same at an early period in the kernel's history, becoming gradually more differentiated with the time of ripening until the size characteristic of each wheat type is attained.

TABLE VII

EFFECT OF DATE OF HARVEST UPON THE VOLUME FRACTION AND SPECIFIC VOLUME OF THATCHER AND MINDUM WHEAT GLUTEN IN 10% SODIUM SALICYLATE

Wheat variety	Harvest date	Protein concentration	ϕ	Volume occupied by 1 g (ϕ/C)
		mg/100 ml	%	*ml*
Thatcher	July 19	2110	14.2	6.73
Thatcher	July 24	2050	17.4	8.49
Thatcher	July 28	2160	20.6	9.54
Thatcher	Sept. 3	2140	19.0	8.88
Mindum	July 28	2140	14.6	6.82
Mindum	July 31	2120	14.2	6.70
Mindum	Aug. 4	2080	13.0	6.25
Mindum	Sept. 3	2040	13.5	6.61

Table VII shows the results obtained by computing the volume fraction [3] and the volume occupied by 1 g of gluten protein when dispersion appeared to have reached completion. The specific volumes of the Thatcher glutens show a distinct increase with the age of the kernel while in the Mindum the differences do not seem to be significant.

Summary and Conclusions

The effect of maturity upon various quality factors of hard red spring and durum wheat was investigated using samples planted and harvested at different dates.

[3] These computations were based upon the equation of Kunitz (J. Gen. Physiol. 9: 715–725, 1926):

$$\eta/\eta_0 = \frac{1 + 0.5\phi}{(1 - \phi)^4}$$

where η = the coefficient of viscosity of the protein sol.
η_0 = the coefficient of viscosity of the dispersions medium.
ϕ = the percentage of the system occupied by the volume of the dispersed phase.

Vitreous kernel content increased with maturity. Test weight increased in the early planted wheats to a maximum a few days before normal harvest, then tended to decrease slightly. Slight decreases with maturity in the late planted series were evident. Flour yield similarly increased in the early planted series but decreased sharply as the grain matured in the late wheats. Weather conditions during the latter part of the ripening period may have affected the results in this instance. Wheat protein was significantly higher in the late-planted wheat and little difference between harvest dates was noticed. In the early planted series, however, protein appeared to be higher in the more mature wheats. Loaf-volume differences were not great, but a slight tendency toward lower values was evident at the end of the ripening period. Absorption consistently decreased with maturity.

Samples of Mindum harvested at four different dates showed increases of protein content, test weight, grade, and macaroni color with age of kernel before cutting. Absorption decreased in a manner similar to that of the bread wheats.

Measurements of viscosity and protein concentration upon dispersions of glutens washed from these wheats in sodium salicylate showed differences in properties that might be attributed to variations in particle size. These differences appeared to be related to ripeness of the kernel when cut and were characteristic of the wheat variety.

The effect of maturity at time of wheat harvest appeared to be more critical in the instance of durum than bread wheats. In fact, in the instance of the late-seeded hard red spring wheats, cutting slightly before normal harvest appeared to improve flour yield and loaf volume.

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APPLICATION OF THE BAKER COMPRESSIMETER TO CAKE STUDIES [1]

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Several devices have been designed for measuring the compressibility of bread and cake crumb. Katz (1917, 1928), Bailey (1930, 1932), Platt (1930), Platt and Kratz (1933), King, Morris and White-

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man (1936), Steller and Bailey (1938), Cathcart (1940), Platt and Powers (1940), and others have described various forms of compressimeters and employed them in cereal chemical investigations. Their principal application has been in studies of bread staling and the effects of variations in formula and procedure on the softness of bread crumb. Several of these workers employed a balance type of compressimeter similar to that proposed by Platt (1930). Platt and Powers (1940), however, described an improved form designed by Dr. J. C. Baker, Wallace and Tiernan Company, which permits convenient measurement of compression at a series of stresses; if desired either variable may be held constant for a given time and the change in the other noted. The instrument is described in *Cereal Laboratory Methods* (4th ed., 1941) and is constructed to measure compression over the range of 0–4 mm and stress over the range of 0–320 g.

Employing the Baker compressimeter, Platt and Powers (1940) studied the effect of several variables on the compressibility of bread crumb; among other factors they noted that the softness of bread crumb increased with the content of sugar, shortening, and milk solids. These observations suggested that the Baker compressimeter might prove a valuable tool in cake studies and led to the investigations reported in this paper.

The primary objective was to study factors affecting the precision of the test and it was desired to obtain cakes which would vary widely in crumb rigidity. Compressibility studies were made with two cake types: one, a modified sponge cake, and the other, a plain yellow loaf-cake. The sponge cake, being light and elastic, was selected as representative of cakes of high compressibility, whereas the yellow loaf-cake, with its fine, close grain represented the less compressible types of cake. In order further to secure cakes differing in crumb softness, variations in formula and mixing procedure were employed. In the instance of the sponge cake, the flour content was varied while, with the yellow loaf cake, various increments of sugar and shortening, respectively, were employed using both the single-stage and creaming methods of mixing. Staling rates of both sponge cake and yellow loaf cake were also followed.

Experimental

Materials: A high-grade cake flour was employed throughout these experiments and the specifications of the other ingredients corresponded to those given in *Cereal Laboratory Methods* (4th ed., 1941) for the cake baking test. The test-baking equipment consisted of a Hobart C-10 mixer equipped with a three-quart bowl, wire whip and paddle, flour sifter, rectangular loaf tins, and a Despatch baking oven (Style No.

135-R) equipped with a rotary hearth. Two sizes of loaf tins were employed, namely the standard cake tin (inside dimensions: top 4 × 8 inches; bottom 3¼ × 7¼ inches; depth 2½ inches) and a smaller tin (inside dimensions: top 3½ × 6½ inches; bottom 2½ × 5½ inches; depth 2¼ inches).

Cake formulas and procedures: The "standard" or reference formulas employed in making the sponge and yellow loaf cakes are given in Table I. In the instance of the sponge cake, the sugar, eggs, and salt

TABLE I

STANDARD OR REFERENCE FORMULAS FOR SPONGE AND YELLOW LOAF CAKE

	Sponge cake	Yellow loaf cake
	g	g
Flour (15.0% moisture basis)	145.0	280.0
Sugar	182.0	300.0
Shortening	—	150.0
Eggs (fresh)	218.0	150.0
Milk solids (dry skim)	5.0	20.0
Baking powder	3.4	10.0
Salt	2.3	3.5
Water	42.0	180.0

were placed in the mixing bowl, warmed to 40°C and whipped on high speed for 3 minutes. The water, preheated to 40°C, was then gradually added over a period of 1½ minutes with the mixer operating on second speed; the whipping was then continued until the batter assumed a characteristic "soft peak" upon removal of the beater (approximately 5½ minutes). The flour, into which the milk solids and baking powder had been incorporated by sifting twice, was next cut in by means of a wide spatula and the mixing completed with five revolutions of the paddle on low speed. The batter was then scaled into either two ungreased standard-size tins with parchment paper bottom-liners, or four of the small tins, and baked at 182°C (±3°) for 25 minutes.

In the single-stage procedure for yellow loaf cake, the skim-milk solids and salt were added to the water, stirred, and placed in the mixing bowl along with the remaining ingredients. After mixing with the paddle for 1 minute on low speed, the bowl was scraped and the batter mixed on second speed for 5 minutes. The bowl was then scraped again and the mixing continued for an additional 5 minutes. The batter (at 25°C) was scaled into two standard-size or four small-size lightly greased tins with parchment paper bottom-liners and baked at 190°C (±3°) for 40 minutes.

In the creaming method for yellow loaf-cake, the sugar, shortening, and salt were creamed for 5 minutes at second speed, the eggs then added slowly over a 5-minute mixing period, and the creaming continued for a further 10 minutes. The flour and reconstituted milk solids were next added in thirds at low speed over a 1½-minute mixing period. The batter was scaled and baked as outlined for the single-stage method.

The yellow loaf cakes were allowed to cool in the pans for 10 minutes and then removed and allowed to cool further on cake racks for 80 minutes; the sponge cakes were cooled (in the inverted position) for approximately 45 minutes before they were removed from the pans and allowed to stand for an additional 45 minutes. The cakes were then weighed, and the volume of the loaf-cakes determined by seed displacement, after which they were wrapped in waxed paper and stored in a constant-temperature cabinet maintained at 26°C.

Measurement of compressibility: The compressibility measurements were made on cake sections 1½ inches square and 1 inch thick accurately cut with the aid of a miter box. The cake was first cut into slices 1½ inches thick, the end slices discarded, and a section 1½ × 1 inch cut from the central portion of each of the remaining slices (4 in the standard size and 3 in the small-size cakes), the long dimension being parallel with the short axis of the cake. Each section thus obtained was centered under the 1½ inch-square plunger of the compressimeter and compression (strain) readings taken for a series of stresses. Because of the mechanical advantage of the lever actuating the plunger, the stress on the cake section is ten times the scale reading.

Effect of variations in flour content on compressibility of sponge cake: The effect of variations in the amount of flour, a so-called toughening ingredient, on the compressibility of sponge cake was investigated by making measurements 24 hours after baking on cakes containing 90, 95, 100, 105, 110, and 115%, respectively, of the weight of flour employed in the reference sponge-cake formula. Two cakes were baked in the standard-size tins from a single mix by each formula in one day, the entire series being replicated four times. In this manner 8 cakes were obtained from each, of which 4 sections were cut, thus providing for a total of 32 compressibility readings for each formula. The mean results, together with a summary of a variance analysis of the data, are recorded in Table II and show that the compressimeter reveals differences in the softness of sponge cake crumb that result from variations in the proportion of flour.

Effects of method of mixing and variations in sugar and shortening content on compressibility of yellow loaf cake: Among other functions, sugar and shortening act as tenderizing agents in cake making and it

TABLE II

EFFECT OF VARIATIONS IN FLOUR CONTENT ON COMPRESSIBILITY OF SPONGE CAKE 24 HOURS AFTER BAKING

Flour as pct of reference formula	Mean compression at stress of		Analysis of variance			
			Source of variation	Degrees of freedom	Mean square for stress	
	10 g	20 g			10 g	20 g
%	mm	mm				
90	1.32	2.69	Formulas	5	2.819**	13.723**
95	0.99	2.25	Days	3	0.338	3.217
100	1.07	2.27	Formulas × days	15	0.252*	1.477*
105	0.88	1.80	Duplicate cakes	24	0.118	0.564**
110	0.63	1.22	Within cakes	144	0.085	0.205
115	0.51	1.00				
Mean	0.90	1.87				
F for formula means	11.21**	9.29**				
Standard error of formula means	0.177	0.215				

* Denotes variance is significant; that is, *F* value exceeds 5% point.

** Denotes variance is highly significant; that is, *F* value exceeds 1% point.

Variance for formulas and days was tested against interaction variance as the error; interaction variance was tested against duplicate error (24 degrees of freedom); variance for differences between duplicate cakes was tested against variance within cakes.

was therefore of interest to determine the effects of varying, individually, the percentages of these ingredients on the compressibility of the cake. For the purpose of this study, no attempt was made to "balance" the cake formula by making appropriate changes in the quantities of other ingredients in order to produce cakes of optimum quality for the particular sugar or shortening level employed. Yellow loaf cakes were baked in the standard loaf tins containing 75, 90, 100, 110 and 125% of the sugar and shortening, respectively, called for in the reference formula. The batters were prepared by the creaming method and also by the single-stage method, in which a rather long mixing time was purposely employed. All levels of one variable employing one mixing method were baked on a single day and each series was replicated 3 times. In this way, 6 cakes representing each mixing method for each sugar and shortening level were available for volume and compression measurements. The mean specific volumes are given in Table III and the mean strain values for stresses of 40, 80, and 120 g. are recorded in Table IV, together with a summary of a variance analysis of the data.

For both mixing procedures, the marked increases in compressibility of the cake crumb with increasing sugar content were highly significant. In striking contrast, the variations in shortening content over the range studied had relatively little effect upon compressibility; for the single-stage method the mean strain values did not differ sig-

TABLE III

EFFECT OF METHOD OF MIXING AND VARIATIONS IN SUGAR AND SHORTENING CONTENT ON THE SPECIFIC VOLUME OF YELLOW LOAF CAKE

Variation in sugar or shortening as pct of reference formula	Mean specific volume			
	Sugar varied		Shortening varied	
	Single stage	Creaming	Single stage	Creaming
%				
75	2.38	2.95	2.60	3.08
90	2.52	2.98	2.59	3.02
100	2.61	2.84	2.65	2.99
110	2.60	2.86	2.52	2.73
125	2.44	2.50	2.55	
Mean	2.51	2.83	2.58	2.96

nificantly, while for the creaming method there was a significant decrease in the strains developed at stresses of 80 and 120 g when the shortening level was increased to 110% of that employed in the reference formula. Values are not included in Table IV for the 125% shortening level (creaming method), as only two cakes were baked, but a mean strain of 1.61 mm was obtained at 120 g of stress. Despite the absence of any significant change in compressibility in varying the shortening from 75 to 100%, the eating quality of the cakes, as reflected by the ease with which they could be chewed, improved as the shortening content was increased. These results clearly emphasize that the compressibility of a cake is not necessarily related to its tenderness or eating quality. To make a far-fetched analogy, a ball of cotton or steel wool is highly compressible, yet has high shearing strength. In tenderness studies, it would be highly desirable to supplement strain measurements with a quantitative shearing test which would simulate the biting action of the teeth.

As anticipated, the creaming method of preparing the batter gave cakes of greater specific volume and compressibility than the single-stage procedure employed here. These differences were probably, in part, the result of excessive mechanical development of the gluten brought about by the prolonged mixing of the flour in the single-stage method.

Effect of staling on compressibility of sponge and yellow loaf cakes: Changes in compressibility of sponge and yellow loaf cakes made by the creaming procedure were studied by means of measurements after 4, 24, 48, and 144 hours of storage at 26°C. The reference formulas were employed and the cakes were baked in the small tins which permitted the production of four cakes from each batter, one of which

TABLE IV

EFFECT OF METHOD OF MIXING AND VARIATIONS IN SUGAR AND SHORTENING CONTENT ON THE COMPRESSIBILITY OF YELLOW LOAF CAKE, 24 HOURS AFTER BAKING

Variation in sugar or shortening as pct of reference formula	Mean compression for indicated stress, formula, and mixing method											
	Stress 40 grams				Stress 80 grams				Stress 120 grams			
	Sugar varied		Shortening varied		Sugar varied		Shortening varied		Sugar varied		Shortening varied	
	Single stage	Creaming	Single stage	Creaming	Single stage	Creaming	Single stage	Creaming	Single stage	Creaming	Single stage	Creaming
	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm
75	0.42	0.54	0.42	0.69	0.55	0.92	0.68	1.41	0.69	1.33	0.97	2.12
90	0.44	0.76	0.45	0.63	0.63	1.36	0.73	1.30	0.79	2.02	0.97	2.06
100	0.52	0.74	0.50	0.70	0.74	1.51	0.78	1.39	1.00	2.33	1.09	2.19
110	0.57	0.90	0.50	0.60	0.86	1.72	0.72	1.13	1.20	2.77	0.96	1.68
125	0.69	1.04	0.49	—	1.15	1.89	0.70	—	1.62	2.89	0.94	—
Mean	0.53	0.80	0.47	0.65	0.79	1.48	0.72	1.31	1.06	2.27	0.99	2.01
F for formula means	7.32*	5.27*	2.49	2.18	18.65**	9.67**	—	6.06*	31.05**	13.47**	2.34	6.97*
Standard error of formula means	0.036	0.082	0.021	0.034	0.054	0.120	0.039	0.052	0.066	0.172	0.032	0.113

ANALYSIS OF VARIANCE

Source of variation	Degrees of freedom	Mean square of compressions for indicated stress, formula, and mixing method											
		Stress 40 grams				Stress 80 grams				Stress 120 grams			
		Sugar varied		Shortening varied		Sugar varied		Shortening varied		Sugar varied		Shortening varied	
		Single stage	Creaming	Single stage	Creaming	Single stage	Creaming	Single stage	Creaming	Single stage	Creaming	Single stage	Creaming
Formulas	4	0.294*	0.851	0.028	0.062	1.322**	3.371**	0.030	0.392*	3.258**	9.542**	0.092	1.263*
Days	2	0.032	0.089	0.190**	0.043	0.011	0.859	0.260*	0.203	0.000	1.953	0.405**	0.304
Formulas × days	8	0.040*	0.162*	0.011	0.028	0.071	0.349*	0.036	0.065	0.105	0.708*	0.039	0.181
Duplicate cakes	15	0.015*	0.054	0.147	0.036**	0.032*	0.091*	0.024	0.070	0.051*	0.229*	0.033	0.110
Within cakes	90	0.012	0.034	0.156	0.012	0.017	0.047	0.020	0.022	0.024	0.100	0.029	0.057

* Denotes variance is significant; that is, *F* value exceeds 5% point.
** Denotes variance is highly significant; that is, *F* value exceeds 1% point.
Variance for formulas and days was tested against interaction variance as the error; interaction variance was tested against duplicate error (15 degrees of freedom); variance for differences between duplicate cakes was tested against variance within cakes. In the shortening series, employing the creaming method, the 125% level was baked on only one day; accordingly the degrees of freedom for the variance analysis are as follows: formulas, 3; days, 2; formulas × days, 6; duplicate cakes, 12; within cakes, 72.

was used for each staling time. Each bake was replicated four times on a single day. The order in which the batters were poured into the tins was recorded and the resulting cakes were distributed at random among the various storage times. Three sections were measured at

TABLE V

EFFECT OF STALING ON COMPRESSIBILITY OF SPONGE CAKE

Time of staling	Mean compression at stress of	
	10 g	20 g
hrs	*mm*	*mm*
4	1.86	3.99
24	0.96	2.06
48	0.61	1.44
144	0.59	1.08
Mean	1.00	2.14
F for staling means	19.33**	38.85**
Standard error of staling means	0.135	0.208

Analysis of variance

Source of variation	Degrees of freedom	Mean square for stress	
		10 g	20 g
Staling times	3	4.235**	20.142**
Replicate error	12	0.588**	1.357**
Within cakes	32	0.081	0.203

** Denotes variance is highly significant; that is, *F* value exceeds 1% point. Variance for staling times tested against replicate error (12 d f); variance for differences between replicate cakes was tested against variance within cakes (32 d f) as error.

TABLE VI

EFFECT OF STALING ON COMPRESSIBILITY OF YELLOW LOAF CAKE

Staling time	Mean compression at stress of				
	20 g	40 g	80 g	120 g	200 g
hrs	*mm*	*mm*	*mm*	*mm*	*mm*
4	0.38	0.63	1.05	1.51	2.73
24	0.30	0.52	0.84	1.20	1.96
48	0.28	0.47	0.78	1.14	1.86
144	0.25	0.45	0.60	0.76	1.17
Mean	0.30	0.52	0.82	1.15	1.93
F for between staling times	3.54*	5.39**	17.71**	29.95**	41.62**
Standard error of staling means	0.029	0.034	0.045	0.061	0.099

ANALYSIS OF VARIANCE

Source of variation	Degrees of freedom	Mean square for stress				
		20 g	40 g	80 g	120 g	200 g
Staling times	3	0.035	0.074*	0.425**	1.142**	4.924**
Replicate error	12	0.011	0.018	0.033	0.083**	0.188
Within cakes	32	0.010	0.012	0.033	0.029	0.092

* Denotes variance is significant; that is, *F* value exceeds 5% point.
** Denotes variance is highly significant; that is, *F* value exceeds 1% point.
Variance for staling times tested against replicate error (12 d f); variance for differences between replicate cakes was tested against variance within cakes (32 d f) as error.

several stresses from each cake so that, in all, 12 measurements were taken for each staling time, stress, and formula. The mean values for the sponge cakes, together with a summary of the analyses of variance, are recorded in Table V, and similar data for the yellow loaf cakes are given in Table VI. The mean compression values were plotted against the staling times and both cake types gave characteristic staling curves; that is, the rate of decrease in compressibility fell off with time of storage. The rate of change was much more rapid for the sponge than for the yellow loaf cake.

Stress/strain relationships: Platt (1930) subjected bread crumb to several different stresses and found that the compressibility varied directly with the magnitude of the stress; in other words, it followed Hooke's law and behaved as a perfectly elastic or compressible body. In studies with sponge cake, Platt and Kratz (1933) made the interesting observation that, while the compressibility of the fresh cakes apparently obeyed Hooke's law, the values at increasing stresses departed more and more from the theoretical as staling progressed. They suggested that the changes in the ratios of the compressions obtained to the weights applied might prove valuable as a measure of the rate of staling.

In studies of the effect of different variables on the compressibility of bread or cake, it is customary to carry out compression measurements at a fixed stress that is selected so as to cause an appreciable compression of the material. As the stresses suitable for a highly compressible material are widely different from those required for a more rigid one, a direct comparison of their compressibilities is not possible. If, however, the materials to be compared are perfectly compressible—that is, if they obey Hooke's law for elastic bodies—a quantitative measure of their relative compressibilities may be obtained readily. The straight lines expressing the relation between compression and the force applied can be extrapolated and the relative compressions read off for a common applied force, or the slopes of these lines may be compared. Moreover, the data for various cakes can be expressed on a comparable unit basis by computing the moduli of compressibility, as has been done by Platt (1940) for bread crumb.

The modulus of compressibility is the stress or force in dynes per square centimeter of surface area which is necessary to produce a unit strain (1 cm). Since in our studies a cake section 1 inch thick $\times$ 1½ inches square was employed, the modulus of compressibility can be computed as follows:

$$C = \frac{\text{stress in grams}}{\text{strain in mm}/10} \times \frac{980.6 \times 2.54}{(3.81)^2}.$$

If the cakes follow Hooke's law, the modulus of compressibility is a constant which is characteristic of the material and entirely independent of the stresses used in carrying out the measurements. As the moduli are expressed on a unit absolute basis, they may be employed not only to secure a numerical measure of the relative compressibilities of cakes made by various formulas and subjected to different treatments but also to compare the compressibility of cakes with that of other elastic or compressible materials of entirely different structure and chemical composition. If, however, cakes do not follow Hooke's law, the modulus values will vary with the conditions of measurement and are valueless for such comparisons.

Since the question as to whether or not the compressibility of cake crumb follows Hooke's law is of both theoretical and practical interest, the stress/strain ratios were calculated for the data of Tables II, IV, V, and VI. These ratios are directly proportional to the corresponding compressibility moduli and are the reciprocals of the slopes of the lines obtained when the strains are plotted on the vertical axis and the stresses on the horizontal axis, with the same scale throughout. The stress/strain ratios are given in Table VII, together with the F values for variance analyses of the data. Nonsignificant F values for the differences between the general mean stresses were obtained in the sponge-cake flour-variation and staling series, and in the loaf-cake series involving variations in sugar and shortening when the batters were

TABLE VII

STRESS/STRAIN RATIOS—GRAMS OF STRESS PER MILLIMETER OF DEPRESSION
(Calculated from Tables II, IV, V, and VI)

Flour variation				Staling data			
Flour as pct of reference formula	Stress in grams		Mean	Staling time	Stress in grams		Mean
	10	20			10	20	
SPONGE CAKE							
90	0.758	0.743	0.750	4	0.538	0.501	0.519
95	1.010	0.889	0.950	24	1.042	0.971	1.006
100	0.934	0.881	0.908	48	1.639	1.389	1.514
105	1.140	1.110	1.125	144	1.695	1.851	1.773
110	1.590	1.640	1.615				
115	1.960	2.000	1.980	Mean	1.228	1.178	1.203
Mean	1.232	1.210	1.221				

	F		F
Formulas	226.80**	Staling times	44.67**
Stresses	Less than error	Stresses	Less than error

TABLE VII—(*Continued*)

Flour as pct of reference formula	Creaming method: Stress in grams 40	80	120	Mean	Single-stage method: Stress in grams 40	80	120	Mean
YELLOW LOAF CAKE—SUGAR VARIATION								
75	74.2	87.4	90.2	83.9	95.2	145.4	173.9	138.2
90	53.0	58.7	59.4	57.0	90.9	127.0	151.8	123.2
100	53.7	53.1	51.5	52.8	76.9	108.1	120.0	101.6
110	44.6	46.5	43.3	44.8	70.2	93.0	100.0	87.7
125	38.4	42.3	41.5	40.7	58.0	69.6	74.1	67.3
Mean	52.8	57.6	57.2	55.8	78.2	108.6	124.0	103.6
		F				*F*		
Formulas		58.51**			Formulas	15.01**		
Stresses		2.34			Stresses	17.15**		
SHORTENING VARIATION								
75	58.0	56.7	56.6	57.1	95.2	145.5	123.7	121.4
90	63.5	61.5	58.2	61.1	88.9	127.0	123.7	113.2
100	57.1	57.6	54.8	56.5	80.0	108.0	110.0	99.3
110	66.7	70.8	71.4	69.6	80.0	93.0	125.0	99.3
125					81.6	69.6	127.6	92.9
Mean	61.3	61.6	60.2	61.1	85.1	108.6	122.0	105.2
		F				*F*		
Formulas		22.91**			Formulas	1.50		
Stresses		Less than error			Stresses	6.35*		

YELLOW LOAF CAKE STALING DATA

Staling time	Stress in grams 20	40	80	120	200	Mean
hrs						
4	52.9	63.5	76.2	79.5	73.3	69.1
24	67.8	76.9	95.2	100.0	102.0	88.4
48	70.7	85.1	102.6	105.3	107.5	94.2
144	79.4	88.9	133.3	157.9	170.9	126.1
Mean	67.7	78.6	101.8	110.7	113.4	
				F		
Staling times				13.69**		
Stresses				8.04**		

* Denotes variance is significant; that is, *F* value exceeds 5% point.
** Denotes variance is highly significant; that is, *F* value exceeds 1% point.

mixed by the creaming method. This indicates that in these instances the compressibility of the cakes obeys Hooke's law.

In contrast, however, the compressibility of loaf cakes baked by the single-stage method employing various quantities of sugar and shortening did not follow this law. Moreover, in the staling of the yellow loaf cake there was an increasing departure from the law as staling progressed. Thus, the differences between the stress/strain ratios for 20 g and 200 g of stress are 20.4, 34.2, 36.8 and 91.5 g per mm of depression for staling times of 4, 24, 48 and 144 hours, respectively. These data thus confirm the observations of Platt and Kratz (1933) in this connection.

It does not appear, however, that the magnitude of these departures from Hooke's law would be of general utility in following staling rates; no such trends are evident in the staling data for sponge cake, whereas they occur in other series where the measurements were made at a constant staling time of 24 hours. Actually, the occurrence and extent of the departures seems to be associated with the magnitude of the individual ratios involved. The more rigid the cake, the greater is the departure from Hooke's law. Thus the more compressible cakes, such as the sponge cakes (flour variation and staling series) and the sugar and shortening variation series baked by the creaming method, appear essentially to follow Hooke's law. In the instance, however, of the more rigid cakes obtained by varying the sugar and shortening in which the single-stage mixing method was used, the departures are evident; moreover, within each series their magnitudes increase with the rigidity of the cakes. For example, in the sugar variation series, the least compressible cake gave a stress/strain ratio of 95.2 at 40 g stress which is 78.7 units less than the stress/strain ratio for this cake at 120 g of stress. As the sugar content of the cakes was increased, they became more compressible and the differences between the stress/strain ratios regularly decreased; the most compressible cake in this series gave a stress/strain ratio of 58.0 at 40 g of stress, which is only 16.1 units lower than the value obtained at 120 g of stress.

The limited data available indicate that the changes in the stress/strain ratios are negatively correlated with the softness or actual compressibility of the cake and are not necessarily related to the staling phenomenon. In other words, the increased differences between the stress/strain ratios for 20 g and 120 g of stress as the yellow loaf cakes underwent staling may simply be a reflection of the increase in the rigidity of the cakes. There may be perhaps a fairly definite value of the stress/strain ratio below which the cake compressibility values will follow Hooke's law. Experiments conducted with sections of varying

thickness showed that the departure from Hooke's law increased as the thickness of the section was reduced.

In the instances where the stress/strain ratios are approximately constant for different stresses, the moduli of compressibility have a real meaning, and a valid comparison of the relative compressibility of the different cakes can be made. Thus sponge cake and yellow loaf cake made according to the reference formula gave mean stress/strain ratios of 0.908 and 52.8, respectively, 24 hours after baking. The corresponding moduli of compressibility are 0.156×10^4 and 9.060×10^4, the sponge cake thus being approximately 58 times more compressible than the yellow loaf cake. These compare with a modulus value of 6.5×10^4 obtained by Platt and Powers (1940) for bread crumb.

Precision of the compressibility test: In analytical determinations there are two main sources of error—those due to variations in the sample and those associated directly with the measurements involved. In many cereal chemical procedures, the various subsamples employed for replicate analyses are relatively homogeneous and the experimental errors largely represent the precision of the test itself. In compressibility measurements, sample variation is undoubtedly the most prominent source of error. In the present study no attempt has been made to evaluate the instrumental error separately from sampling error, since interest in such information would be largely theoretical.

The variance analyses recorded in Tables II, IV, V, and VI show the magnitude and significance of several sources of error. These include the variance due to differences between measurements made in sections cut from one cake, that due to differences between the values for cakes baked from a single mix, that due to differences between days (that is, between the values for batters mixed on different days), and that due to the interaction of main effects (formula variations and time of staling) with days (batters). In about half the cases, the differences between replicate cakes baked from the same batter were significant, but in only 4 out of 14 cases was the variance for between days (batters) significantly greater than the variance within a single mix. In several instances, the interactions of the main effects with days (batters) were significant.

The absolute errors are large in relation to the magnitude of the values involved and the relative errors tend to increase with the compressibility. Thus, the relative errors were much higher for the sponge than for the yellow loaf cakes. For the staling data, errors were computed separately for each of the staling periods but only the pooled errors for all staling times are recorded in Tables V and VI. The coefficients of variability of the treatment means (based on 12 values) for 10 g of stress in the sponge-cake staling series were 12.7,

12.1, 7.4 and 7.6% for 4, 24, 48 and 144 hours, respectively. Similar but less pronounced trends were observed for the readings at 20 g stress and also in the loaf-cake staling series.

Readings may conveniently be made with the Baker compressimeter at a series of stresses and the question arises as to the most appropriate stress to employ in order to secure the maximum precision. The data clearly indicate that, as specified in *Cereal Laboratory Methods* (4th ed., 1941), a stress which gives compression values close to the maximum which the instrument is capable of recording should be used. In all studies except that involving a variation of the flour content of sponge cake, the *F* values for the variable under study increased with the stress employed; for example, the *F* values for the differences in the compressibility of yellow loaf cake at various staling times increase from 3.54 for the readings at 20 g of stress to 41.62 for those taken at 200 g of stress.

Summary

The Baker compressimeter was employed in a study of the precision of cake compressibility measurements at various stresses and of the effects of staling, formula variations, and mixing procedures on the compressibility of sponge and yellow loaf cakes.

Compressibility of sponge cake decreased as the flour content was increased. Compressibility of yellow loaf cakes increased progressively as the sugar in the formula was increased from 75% to 125% of the sugar employed in the reference formula. More compressible cakes were obtained when the batters were mixed by the creaming method and the changes in compressibility with variations in sugar content were greater when the batters were mixed by the creaming method rather than by the single-stage method.

Shortening variations of 75% to 125% of the shortening employed in the reference yellow loaf cake formula were without significant effect upon cake compressibility when the batters were mixed by the single-stage method. The creaming procedure gave higher compressions for corresponding formulas and there was a slight decrease in cake compressibility when the shortening was increased above that specified in the reference formula.

Staling of sponge cake as measured by changes in compressibility occurred more rapidly than that of yellow loaf cake.

Stress/strain ratios for the various stresses showed that the compressibility of sponge cake and yellow loaf cakes prepared by the creaming method tested 24 hours after baking obeyed Hooke's law. With the less compressible yellow loaf cakes obtained in the formula variation and staling studies, there was an increasing departure from

Hooke's law as the compressibility decreased. Moduli of compressibility of sponge and yellow loaf cakes (creaming method) made by the reference formula and tested 24 hours after baking were 0.156×10^4 and 9.06×10^4, respectively, indicating that the sponge cake was 58 times more compressible than the yellow loaf cake.

Relatively large errors are involved in compressibility measurements. These include the variance due to differences between (1) measurements on individual sections cut from a single cake, (2) cakes baked from a single mix, (3) cakes made from different batters baked on different days, and (4) the interaction of main effects (formula variations and staling times) with days or batters. The magnitudes and significance of these sources of error were determined.

Increased precision is obtained by making the measurements at the maximum stresses at which compression values may be read with the instrument.

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THE EFFICIENCY OF THE PRODUCTION OF ETHANOL FROM STARCHY SUBSTRATES

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The current extensive use of starchy materials for the manufacture of industrial ethyl alcohol (about 400 million bushels of grain per year) creates new interest in the efficiency of the process. Each manufacturer has his own criterion of conversion efficiency, and while the numbers are of comparative value, they do not give an adequate picture of the absolute efficiency obtained. Since raw material cost is ordinarily three-fourths of the total cost of the alcohol manufactured, the question of efficiency of conversion is a most important matter.

Statements about the efficiency of the conversion of starchy farm crops to ethanol are apt to be rather confusing because there are so many kinds of units used. The yield may be stated in terms of gallons of 190° proof alcohol, 200° proof alcohol, or in proof gallons, which is the same as wine gallons of 100° proof. The raw material unit may be a bushel, one hundred pounds, or a long or short ton, and may be on the basis of any of several moisture contents. Bushel weights are variable, not only from one material to another, but even in a single material. Even on a moisture-free basis, the carbohydrate content of any one raw material is variable. In some cases yields are calculated on the basis of cleaned grain and in others on the weight of grain brought into the plant. Sometimes the malt is included as a part of the raw material charged to process; in other cases the alcohol expected from the malt is deducted from the total alcohol produced and the balance credited to the corn or other grain. Several procedures are followed with regard to the alcohol produced from the inoculum.

This situation has led to an attempt to state yields in terms of percentage of theoretical. The raw material may be analyzed by one of the standard methods for starch estimation, and since the weight of grain charged to process is accurately measured, the amount of starch processed may be estimated, or the mash ready for inoculation may be similarly analyzed. Then the theoretically possible alcohol yield may be calculated on the assumption that one mole of dextrose yields two moles of ethanol. Since the actual production of ethanol can be estimated quite accurately by measurement of specific gravity or refractive index of the distillate from a measured volume of the fermented mash,

[1] A part of the research herein reported was done by the author in the laboratories of the Department of Agricultural Chemistry, University of Idaho, Moscow.

it is thus possible to calculate an efficiency which is generally regarded as approaching an absolute value.

Vernon and Metzner (1941) describe a procedure now commonly employed in calculating the "true fermentable carbohydrate" content of grains. This value is arrived at by estimating total carbohydrate content, called the "starch" content, by acid hydrolysis, and then subtracting the unfermentable carbohydrate content, which is established by estimation of the "pentosan" present in the grain by a rapid bromine method. Data are presented for six samples of corn. Following are the mean values:

A—"Starch" content	70.57%
B—"Pentosan" content	6.22%
C—"Starch true fermentable substance"	64.35%
D—Theoretical alcohol yield	6.17 proof gal/bu
E—Assuming 88% of theory as maximum	5.43 proof gal/bu
F—Actual alcohol yield	5.16 proof gal/bu
G—Fermentation efficiency F(100)/E	95.05%

It should be noted that the calculated efficiency is based upon the assumption that 88% of theoretical is the highest yield attainable and this is used as the base for calculating the efficiency. Actually, the efficiency of the process is only 5.16 (100)/6.17 = 83.6%.

When grains are used for alcohol manufacture in a modern plant, all of the residual solids are recovered as a valuable byproduct. These residual solids should contain all of the protein, minerals, fat and fiber of the grain processed, plus the starch, dextrins, and sugars not converted to alcohol, plus the yeast generated in the process, plus the nonvolatile byproducts of fermentation such as glycerol and succinic acid. Since 162 g of starch plus 18 g of water should yield 92 g of ethanol plus 88 g of CO_2, each gram of alcohol produced represents 1.761 g of starch. Then the theoretical yield of byproduct feed is the difference between 100 and 1.761 times the ethanol yield per 100 g of dry matter charged to process.

When this analysis is applied to the published data on alcohol and residual solids recovery, an important loss in process is revealed, as shown in Table I. In the case of corn, only 85.5% of the dry matter charged to process is accounted for as residual solids and ethanol. During process there has been a loss of 14.5% of the dry matter.

Losses of this same character can also be shown in laboratory-scale fermentations. Thus, in a typical case, 120 g of high-quality corn of 13.0% moisture content and 15 g of rye of 14.3% moisture content were mashed with 900 ml of water. This mash was cooked 60 minutes at 120°C, cooled to 60°C, and saccharified with 15 g of malt of 11.5% moisture content, the saccharifying mash being held 90 minutes at 60°C. The mash was then cooled to 30°C and inoculated with 10 ml

of a culture of a good distiller's yeast in beer wort containing, originally, 14.0 g of dry matter per 100 ml. The total dry matter per flask was, therefore, 120 (0.870) + 15 (0.857) + 15 (0.885) + 1.4 = 131.9 g. Three flasks were set in this manner and were incubated 84 hours at 30°–32°C.

TABLE I

CONVERSION EFFICIENCIES IN PRESENT COMMERCIAL OPERATIONS

Raw material	Yields, g/100 g total dry matter processed		
	Ethanol (A)	Dry residual solids (B)	Efficiency (A) (1.761) + (B)
Corn + malt (Christensen *et al*, 1936)	32.9	27.4	85.4
Corn + malt (Cooley, 1938)	34.2	28.0	88.3
Corn + malt (Jacobs and Newton, 1938)	33.7	24.5	83.9
Wheat + malt (Jacobs and Newton, 1938)	33.1	28.7	87.1
Barley + malt (Jacobs and Newton, 1938)	31.0	34.6	89.2
Rye + malt (Jacobs and Newton, 1938)	30.9	29.0	83.4
Potatoes + malt (Jacobs and Newton, 1938)	33.9	15.4	75.1
Corn + malt (Shepherd *et al*, 1940)	31.0	29.7	84.3
Corn + malt Mean above	33.0	27.4	85.5

Two 200-ml samples were taken from each flask at the completion of fermentation, one for distillation and one for measurement of residual solids. The ethanol was estimated by the measurement of the specific gravity of 100 ml of distillate from the 200-ml sample, to which 100 ml of water was added prior to distillation. The mean ethanol content was 42.8 g per flask, or 32.4 grams per 100 grams of dry matter charged to process. The yield of residual solids was 38.6 g, dry basis, per flask, or 29.3 g per 100 g of dry matter charged to process.

The overall conversion efficiency realized in these fermentations was 32.4 (1.761) + 29.3 = 86.5%. The loss in process was 13.5%.

Beresford and Christensen (1940) reported losses in fermentations of corn mash by this same or a similar procedure ranging from 11.8% to 15.9%, the loss being greater from low than from high quality corn. Similar losses were found in the fermentation of white potatoes, grain sorghums, rye, barley, wheat, cassava and other starchy materials.

Taylor (1939) made a study of this loss in the fermentation of potatoes and found that there was a steady increase in the discrepancy between the original carbohydrate content of the mash and the sum of the carbohydrate and the carbohydrate equivalent of the ethanol at intervals during the period of fermentation. Measurements were made by one of the standard Fehling methods.

The character of the material lost can also be established by comparing the analyses of raw material and of residual solids. In Table II are shown the results of such a comparison, the data being taken from

the fermentation described above. The character of the loss is obvious; it is in the nitrogen-free extract portion of the grain. The loss amounts to 13.5 (100)/80.4 or 16.8% of the total nitrogen-free extract.

Assuming that all of the nitrogen-free extract is convertible to alcohol, which is of course not exactly true, the conversion process, including saccharification and fermentation, is only 83.2% efficient. This is nearly exactly the efficiency calculated from the data of Vernon and Metzner (1941).

TABLE II

AN ACCOUNTING OF THE COMPONENTS OF THE GRAIN PROCESSED

Component	Present in the dry grain processed	Present in the dry residual solids	Represented by the ethanol produced	Accounted for in the products
	g	g	g	g
Protein, crude	10.9	10.5	—	10.5
Fat, crude	4.5	4.3	—	4.3
Ash	1.6	1.6	—	1.6
Fiber, crude	2.6	2.7	—	2.7
N-free extract	80.4	10.2	57.2	67.4
Total	100.0	29.3	57.2	86.5

It has already been determined in this laboratory that this carbohydrate loss is evidenced by a large production of CO_2 and water during distillation and the drying of residual solids, but as yet little is known about the actual mechanism preceding these operations. It is indicated, however, that practically all of the starch not converted to fermentable sugars in the saccharification process and subsequently converted to alcohol finally is lost, probably as CO_2 and water. Studies are now in progress to supply further information about this matter. It may well be that the mechanism is something like that occuring in stored blackstrap molasses.

Summary and Conclusion

By measuring yields of both alcohol and dry residual solids per 100 g of dry matter charged to the fermentation process, it is possible to establish a useful index of the fermentation efficiency.

In the present orthodox process for the manufacture of ethyl alcohol from grains, there is a loss of carbohydrate, probably as CO_2 and water, amounting to about 17% of the total carbohydrate, as starch charged to process. The present process is thus only about 83% efficient as regards carbohydrate utilization, not 92 to 95% as commonly reported.

In addition to a statement of yields on the basis of dry matter charged to process, it may also be desirable to state the alcohol yield in terms of the dry weight of the principal raw material processed. This

can be done by subtracting from the total alcohol produced in the fermenter the amount of alcohol expected from the inoculum, the saccharifying agent, and any other minor constituents, crediting the balance to the major constituent. Obviously this yield must not be used in calculating the overall conversion efficiency.

With our knowledge of carbohydrate estimation on the present unsatisfactory basis, it is not recommended that yield data be stated in terms of percentage of theory.

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MICRO TESTS OF ALIMENTARY PASTES II. EFFECTS OF PROCESSING CONDITIONS ON PASTE PROPERTIES [1]

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The investigations described in this paper were undertaken primarily with the object of improving the reproducibility of results obtained with the micro test for alimentary pastes developed by Fifield, Smith, and Hayes (1937). In a previous paper (Cunningham and Anderson, 1942) new techniques and apparatus were described, including a photometric measurement of the opacity (optical absorption coefficient) of the paste. During the course of these developments, the effects on paste properties of varying the processing procedure were

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studied, at first for purposes of selecting those stages at which close control was required to obtain reproducible results, and later with the object of obtaining a background of information which would be useful in studying the utility of the micro test for assessing new varieties of durum wheat. The more important results of these studies are recorded in this paper.

The micro tests consist of mixing 50 g of semolina with water to form a stiff dough, folding and sheeting this a number of times, cutting a small disk ($2\frac{3}{16}$ inches in diameter) from the sheet of dough, pressing this and drying it between sheets of paper. The opacity of the dried disk is then measured with a photometer, and its color is determined by matching against Wallace and Tiernan disks (Baker, Parker, and Freese, 1933). The photometric measurement of opacity is rapid, objective, and extremely precise, whereas the determination of color is tedious, subjective, and lacking in precision. In studying the effects of processing conditions on one semolina it has been found that the color quality of the finished disk is inversely related to its opacity. Accordingly, the change in opacity has been used as a criterion of change in paste properties throughout this investigation, and it is not considered necessary to complicate this paper by introducing data on the color of the disks. Indeed, such measurements were not always made because of the time required, and because reporting them would involve dealing with four additional figures for each disk, namely, the percentages of yellow, red, white, and black required to match the color of the disk. The effect on disk color of modifying the more important processing conditions will be described in a further paper.

The following processing factors have been studied: absorption; time and temperature of mixing; length of rest period prior to sheeting; number of sheeting and folding operations; pressure, press temperature, and time in press; and time, temperature, and humidity during drying of the disk. It was soon apparent that the study of the effects of processing was complicated by interactions between various factors; *e.g.* the effect on one semolina of a given change in pressure is not constant but varies with the absorption and amount of mixing. Accordingly, it was necessary to use factorial designs in most experiments.

Theoretically it would appear advantageous to study all factors simultaneously, but with 12 factors this would require 4,096 disks (over 12 months' work) even if only two levels of each factor were studied without replication. In practice, and because ideas for the investigation were developed piecemeal, it was necessary to split the investigation into a number of smaller and more manageable experiments. As the study developed, many of the preliminary experiments were superseded by others of better design. Accordingly, no attempt is made to

present all the work which has been undertaken and only the more significant experiments are described.

The largest experiment involved the simultaneous study of seven factors at two levels of each. One semolina was used and duplicate disks were made for each treatment. The major portion of the paper deals with this experiment. However, before proceeding to a consideration of the effects of these seven factors, it seemed best to present the results of certain minor experiments dealing with the remaining five factors. Data from some other experiments are also introduced where these serve to elucidate the results of the major experiment.

An extension of the investigation obviously involves a study of the differential effect of processing methods on different semolinas. An initial study of this kind has been undertaken, in which both opacity and color measurements were made, but it seemed best to deal with this investigation in a separate paper.

Materials and Methods

Semolinas milled from the following seven samples of durum wheat were used in the investigation: (A) 1 C.W. Amber durum, 1941 crop, composite No. 1; (B) 2 C.W. Amber durum, 1941 crop, composite No. 1; (C) 3 C.W. Amber durum, 1941 crop, composite No. 1; (D) 1 C.W. Amber durum, 1941 crop, composite No. 2; (E) 2 C.W. Amber durum, 1941 crop, composite No. 2; (F) Mindum, 1940 crop; (G) Golden Ball, 1940 crop.

For general information on the methods and equipment used in preparing the disks of alimentary paste, the reader is referred to a paper by Fifield, Smith and Hayes (1937). The modified equipment and technique used in this laboratory were described in the first paper in this series (Cunningham and Anderson, 1942). The latter paper also contains a detailed description of the method used for determining the opacity of the disks, and a preliminary discussion of the relation between opacity and color characteristics.

Effects of Press Temperature, Rest Period, and Drying Conditions on Absorption Coefficients

Preliminary experiments which need not be described had demonstrated the relative importance of most of the processing factors. They indicated that it would not be necessary to provide close control of press temperature, nor of air humidity during drying, and that rest period and drying time could be readily standardized. These conclusions were checked by means of the three experiments described in this section.

Press temperature: The Carver press is supplied with plates having built-in electrical heating units. The temperature of these can be controlled by means of a rheostat and the metal press bowl and plunger can be readily conditioned by lagging them and leaving them between the heated plates.

Disks pressed at 27°C were compared with disks pressed at 37°C in an experiment involving the preparation of 48 disks. Replication was obtained by using a factorial design in which the two temperature levels were combined with six levels of pressure (600, 800, 1,000, 1,200, 1,400 and 1,600 pounds per square inch) and two levels of mixing time (40 and 100 seconds). Semolina No. 2 was used in this experiment. There were thus prepared 24 sheets of dough, representing each of the 24 combinations of conditions, and duplicate disks were cut from each sheet of dough.

For purposes of the present discussion, only the effect of press temperature need be recorded. The 24 disks pressed at 27°C had a mean optical absorption coefficient of 3.92, while the disks made at 37°C had a mean value of 3.98. Statistical analysis showed that the difference between these values was not significant, and that significant interactions did not occur between press temperature and pressure, or between press temperature and mixing time. In this connection it may be noted that these other two factors have a relatively large effect on the absorption coefficient and are thus likely to bring to light significant interactions with other factors if these occur.

As a 10°C difference in press temperature has a negligible effect on paste properties, it was concluded that it would not be necessary to control the temperature of the press, and that it would be satisfactory to operate it at room temperature. This conclusion may seem at variance with that of Binnington and Geddes (1936) who found it advisable to control the temperature of the press used for making macaroni under laboratory conditions. It should be noted, however, that in forcing a dough through a die with a plunger operating at constant speed, the pressure exerted on the dough is directly proportional to its consistency. Since pressure has a large effect on paste properties, it is obviously necessary to control consistency closely in making macaroni, and this involves control of the dough temperature through regulation of the press temperature. In the micro test pressure is applied independently of the consistency of the dough. Accordingly, in the micro test there is no need to control consistency, by controlling temperature, in order to standardize pressure.

Rest period: In commercial practice and in the laboratory macaroni-making process of Binnington and Geddes, the rest period prior to pressing appears to be necessary in order to adjust the temperature,

and thus the consistency and plasticity of the dough, so that it will react uniformly to the pressure exerted by a ram driven at constant speed. In the micro test of Fifield and coworkers, a rest period of 30 minutes is obtained by rolling the dough into a ball after sheeting 20 times and allowing it to remain in a beaker tightly covered with waxed paper. After the rest period it is necessary to sheet the dough again. These authors note that neither the temperature nor time of the rest period has an important effect on the finished disks. For this reason, and because pressure is independent of dough temperature in the micro test, it appeared that the rest period might well be eliminated.

Rest periods of 0, 15, 30 and 45 minutes were investigated in an experiment involving the preparation of 128 disks. Replication was obtained by using, in addition, two drying temperatures (16° and 26°C), two drying times (2 and 3 days), and four semolinas (A, B, C and D). Duplicate disks were made for each of the 64 combinations of semolinas and treatments.

The mean absorption coefficients for the four rest periods were as follows: 0 min., 2.64; 15 min., 2.54; 30 min., 2.50; and 45 min., 2.51. While statistical analysis showed that the effect of the first 15-minute period was significant, it also showed that further increases in the rest period had an insignificant effect, and that no significant interactions exist between rest period and semolinas, or between rest periods and either of the other two factors. The small improvement in dough properties caused by a 15-minute rest period can be obtained in other ways, *e.g.*, by increasing pressure. There thus appears to be no good reason for using a rest period in the micro test, and some simplification and speeding up of the process results when the rest period is eliminated.

Drying conditions: In the better commercial plants the rate of drying of macaroni is carefully controlled by controlling the temperature and humidity of the air. It is necessary to use a falling humidity gradient so that the loss of moisture from the surface of the macaroni is little greater than the rate of transfer of moisture from the interior of the paste to the surface. If the drying is too rapid, mechanical faults occur in the macaroni; cracks develop and the tubes may split and curl. On the other hand, if drying is too slow the goods may sour or develop mold growths.

In making micro disks it has frequently been observed that disks break if drying conditions are not controlled. This appears to occur if too steep a moisture gradient is created between the surface and the interior of the disks, or between the edges and the center. Thus if disks are dried between sheets of cellophane so that the edges dry more rapidly than the center, then chipping of the edges and breaking of the disks occur. Again, if disks are partially dried between bond paper,

removed while their moisture content is still relatively high, and left in a room at low humidity, the surfaces of the disks dry out more quickly than the body of the paste and the stress set up by the sharp moisture gradient causes the disks to break. This does not occur if the moisture content is reduced slowly. The reverse effect can also be obtained by exposing dry disks to high humidities, when the surface takes up moisture too rapidly and the disk again tends to break. On the other hand, if the moisture content of the disk is increased slowly, no cracking occurs.

So far as the writers can determine the importance of drying conditions relates primarily to the mechanical properties of the disks. Disks which do not break can be prepared under a fairly wide range of drying conditions, and have relatively uniform opacity and color characteristics. A full investigation has not yet been undertaken because experience leads us to believe that in the micro test the matter is not so important as it might appear from consideration of the care required in drying macaroni under commercial conditions. However, the results of three experiments dealing with drying conditions are reported below.

The first of these is that described under the previous heading in which two drying temperatures and two drying times were studied in addition to different rest periods. One set of disks was dried at 26°C and a relative humidity of 20%, and a second set was dried simultaneously at 16°C and a relative humidity of 37%. After two days, half the disks were removed and their opacity measured, and the remaining disks were removed and measured after three days.

The following results were obtained for absorption coefficients: at two days, 2.45 for 16°C and 2.65 for 26°C; at three days, 2.48 for 16°C and 2.60 for 26°C. Statistical analysis showed that the effect of the additional day was just significant, but that no significant interactions occurred between time and temperature, or between these and the other two factors included in the experiment, namely, rest period and semolinas.

On the other hand, the difference of 0.16 between drying temperatures was significant. It should be observed that this difference, resulting from a variation of 10°C in drying temperature, is slightly below the amount of change in opacity or color which can be observed by eye. Unfortunately the moisture content of the finished disks was not measured in this experiment but results described below indicate that the difference cannot be wholly ascribed to possible differences in moisture content. In any event it was decided that since it was convenient to dry the disks in an available room in which temperature was controlled at 26°C this change in procedure might well be adopted.

In a further experiment disks made from semolina D were dried in quadruplicate for 48 hours at 26°C, at relative humidities of 33, 65, and 76%. The disks from the two higher humidities were still too wet to handle, 18.0% and 19.6% moisture, as compared with 14.5% for disks dried at 33% rh. Accordingly, all disks were dried an additional 20 hours at room humidity (57% rh). The disks were then weighed and measured, and opacity was determined. These determinations were repeated at 92 and 232 hours, measured from the beginning of the drying period. The data are shown in Table I.

TABLE I

EFFECT OF RELATIVE HUMIDITY DURING DRYING ON OPACITY

Relative humidity during first 48 hrs.	Property	Properties of paste after				Total change from 68 hrs.
		48 hrs.	68 hrs.	92 hrs.	232 hrs.	
%						
33	Moisture content (%)	14.5	12.9	12.5	11.4	1.5
	Abs. coeff.	—	2.82	2.84	2.92	0.10
65	Moisture content (%)	18.0	13.6	12.8	11.5	2.1
	Abs. coeff.	—	2.81	2.84	2.91	0.10
76	Moisture content (%)	19.6	14.2	13.0	11.5	2.7
	Abs. coeff.	—	2.79	2.81	2.90	0.11

It will be observed that a difference of 1.3% of moisture existed between the first and last sets of disks at 68 hours, but that the difference in the absorption coefficient was only 0.03, a negligible amount. A slight difference in the absorption coefficient persisted at 232 hours, by which time all disks had reached essentially the same moisture content. From 68 to 232 hours the first set of disks lost 1.5% of moisture (last column of data), the second set lost 2.1%, and the third set lost 2.7%. Yet all three sets showed essentially an equal increase in absorption coefficient. This amounted to 0.10 to 0.11 unit. It might be mentioned that from 68 to 232 hours the average thickness of the disks decreased from 0.213 to 0.210 cm. These data indicate that the initial relative humidity at which the disks are dried, and the final moisture content of the disk, had very little effect on the absorption coefficient. On the other hand, it appears that there is an aging effect of appreciable magnitude if the disks are kept for some time.

In a further experiment the moisture content of 14 aged disks was gradually increased by exposing them to atmospheres of increasing humidity. The mean moisture content was thus raised from 11.0% to 14.5%. The mean disk thickness increased from 0.212 to 0.214 cm, and the absorption coefficient decreased from 2.78 to 2.74. A very small change in disk quality, far less than can be observed by eye, was thus obtained for a change of 3.5% in moisture content.

As a result of these experiments it was concluded that sufficiently uniform results for all practical purposes could be obtained by drying disks at a controlled temperature of 26°C for two days and making measurements of the absorption coefficient and color immediately. In this connection it should be noted that drying conditions will always be uniform for a batch of disks made on any one day, and in the micro test 16 sets of duplicate disks can be prepared each day. In experiments in which more than 16 semolinas are to be compared, uncontrolled variations in drying conditions may contribute to the inter-batch error, but this error can generally be controlled by a suitable statistical design.

While it is realized that the experiments described in this section do not serve to elucidate the effects of drying conditions on paste properties, they indicate that these are not large. Accordingly, it seemed preferable to press forward with other phases of the investigation before attempting a more comprehensive study of drying conditions.

Effects of Absorption, Mixing, Sheeting and Pressure on Absorption Coefficients

The effects of seven processing factors were studied simultaneously in a factorial experiment involving two levels of each factor. The factors and levels were as follows: (A) absorption, 28% and 32%; (B) mixing temperature, 26° and 33°C; (C) mixing time, 40 and 100 seconds; (D) sheeting, temperature of rolls, 24° and 37°C; (E) sheeting, number of times, 5 and 20; (F) pressure on gauge, 800 and 1600 lbs per square inch; (G) time in press, 3 and 8 minutes. Semolina D was used in this experiment. There were thus 128 treatments: one dough was made for each treatment and duplicate disks (total number, 256) were prepared and measured. Sixteen doughs (32 disks) were prepared each day so that eight days' work was required for processing, together with an additional three days for drying and measuring the last batch of disks.

Theoretically, a complete randomization of treatments would have been desirable in this experiment. In practice this ideal could not be achieved. It could have been attained if two mixers and two sets of sheeting rolls, operated at different temperatures, had been available; or if the temperatures of the mixer and rolls could have been changed rapidly in the interval between the processing of one dough and the next. Neither of these solutions was considered practicable.

Temperatures could be changed at half-day intervals (*i.e.*, overnight or during the lunch period) so that it was necessary to fit the experiment to 16 half-day periods in each of which treatments could be

made under one of the four possible combinations of two mixing temperatures and two sheeting temperatures. Four half-day periods were required to complete the 32 treatments (the combination of the remaining five factors at two levels of each) required at each temperature combination. These 32 treatments were allotted at random over the four half-day periods, a different randomization being used for each temperature combination. The 16 half-days of work were then undertaken in random order.

It was subsequently found that this design was not adequate. Owing to unintentional confounding it did not provide an estimate of error for the comparison of the temperature factors (B and D) or of the interaction between them (B × D). This difficulty could have been overcome by careful confounding of some of the higher interactions within batches, but this would have involved a complex design beyond the ability of the writers to devise. The design used is satisfactory provided that the inter-batch error (*i.e.*, the error between half days) is negligible. No internal test of this point can be made, but on the basis of other experiments the writers believe that the inter-batch error is not large enough to introduce a serious bias. On theoretical grounds, a straightforward analysis of the variance of the results of this experiment, disregarding the possible bias introduced by unintentional and inextricable confounding, cannot be defended. In practice, the writers believe that it is safe.

Effects of Main Factors

The effects of the main processing factors on the absorption coefficient are shown in the upper part of Table II. The results of experi-

TABLE II

EFFECT OF PROCESSING CONDITIONS ON ABSORPTION COEFFICIENTS OF ALIMENTARY PASTE

Variable	Range	Absorption coefficient	
		Range	Difference
A Absorption (%)	28–32	4.63–3.75	0.88
B Mixing temperature (°C)	26–33	4.26–4.11	0.15
C Mixing time (*seconds*)	40–100	3.36–5.02	1.66
D Sheeting temp. of rolls (°C)	24–37	4.21–4.16	0.05
E Sheeting (*number of times*)	5–20	4.09–4.28	0.09
F Pressure (*lbs./sq. in.*)	800–1600	5.50–2.87	2.63
G Time in press (*minutes*)	3–8	4.42–3.96	0.46
Press temperature (°C)	27–37	3.92–3.98	0.02
Rest period (*minutes*)	0–15	2.64–2.54	0.10
Drying conditions:			
Temperature (°C)	16–26	2.46–2.62	0.16
Time (*days*)	2–3	2.55–2.54	0.01
Relative humidity (%)	33–76	2.82–2.79	0.03

ments with other processing factors, described in the previous section, are summarized in the lower part of Table II. These are not discussed a second time, but are presented for ready comparison with data for the seven processing factors studied in the present experiment.

The results of the analysis of variance are shown in Table III. This was not carried further than the second-order interactions, and those of the first- and second-order interactions yielding mean squares lower than that of the remainder were subsequently recombined with the remainder so as to increase the number of degrees of freedom available for the test of significance.

Absorption: The levels of water absorption studied represent the extremes of the range of working consistency for the semolina used. The optical absorption coefficient decreases with increasing water absorption; *i.e.*, the disks become less opaque and thus look yellower as the water absorption is increased. Other experiments, in which a greater number of levels of water absorption were used, show that the relation is not linear, but that opacity decreases more rapidly for a given increase in water absorption at the lower end of the range than at the higher end.

Binnington and Geddes, working with a paste extruded from a press in a long strip, found that changes in absorption from 27% to 32% had a marked effect on paste color as measured by a spectrometric determination of reflected light. With increasing absorption, "brightness" decreased and "purity" increased, while the dominant wave length remained practically unaltered. Fifield *et al.* noted only that the quantity of water added must be adjusted to form a dough of proper consistency.

As has been mentioned previously, the pressure exerted on a dough extruded through a die, as in the commercial process or in making macaroni in the laboratory, depends on the consistency of the dough. This in turn depends mainly on the water added. It should be noted, however, that in commercial practice an attempt to lower the opacity and increase the color of the paste by increasing the water absorption would be offset, at least in part, by the reduction in the pressure under which the dough would be extruded. This conclusion is based, of course, on the assumption that the ram in the press is driven at constant speed.

It is clear that in order to obtain reproducible results in the micro test, water absorption must be closely controlled. The choice of the proper absorption level for different semolinas is not easy to decide. For reasons discussed above, it appears that constant consistency must be used in laboratory macaroni making by the extrusion process, but this is not easily determined, and requires additional semolina for the

preliminary tests by "cut and try" methods. While further investigation of this point is required, the writers have formed the tentative opinion that for the micro test it will prove practical to use a constant absorption of 30% for all semolinas. This simplification can be achieved because the micro test has the advantage of permitting independent control of the pressure applied to the paste and paste consistency. In the macro test independent control of pressure is not obtained and it is therefore important to adjust dough consistency to a predetermined level.

Mixing temperature: The effect (0.15 unit, see Table II) of a 7°C increase in mixing temperature can be readily determined by the photometer, but is barely apparent even to a trained eye. The opacity of the disks tends to increase with increasing mixing temperature. Results obtained in other experiments, in which a wider range and more levels of mixing were studied, indicate that the relation between opacity and mixing temperature is not linear under all conditions. However, the major irregularities occur at temperatures lower than those used in this present investigation.

In order to obtain maximum reproducibility in the micro test, it seems advisable to control temperature during mixing by surrounding the mixer with a thermostatically controlled water bath, and this has been done (Cunningham and Anderson, 1942). If this practice is not followed the mixer gradually heats up as a series of doughs is mixed. There is thus introduced an intra-batch (intra-day) error, over and above any inter-day error which might result from the effects of changes in room temperature on a mixer not equipped for temperature control.

In this laboratory the doughs are now mixed at 30°C. This temperature was chosen quite arbitrarily as sufficiently above room temperature to make control feasible with a heated water bath.

Mixing time: Table II shows that the amount of mixing the dough receives has a relatively large effect on the opacity of the disks made from it. An increase in mixing time increases opacity and thus decreases the apparent yellowness of the disk. It will be observed that a wide range of mixing time was selected for the experiment, so that the results obtained cannot be said to be at great variance with the comment of Binnington and Geddes, that minor variations in mixing time have no appreciable effect on paste properties. However, it should be noted that by using the precise measurement of the absorption coefficient as a criterion of quality, the effects of small variations in mixing time can be demonstrated, even though these cannot be detected by color matching.

The technique of Fifield *et al.* involves mixing for one minute in a hand-operated mixer. In view of the effect of variations in the amount of mixing, it appeared that this technique could be improved by using a motor-driven mixer. In this laboratory the mixing process is also timed with a stop-watch and every attempt is made to develop a strictly standardized technique.

A mixing time of 40 seconds has been tentatively adopted as standard. It was selected partly because short mixing yields more transparent and yellower disks, and partly because evidence has been obtained which shows that with increases in mixing time the dough becomes more sensitive to changes in other processing factors. Careful examination of series of disks leads to the belief that longer mixing increases opacity chiefly because it increases the number of minute air bubbles incorporated in the paste. As an exaggerated simile, it can be said that the process is similar to that of beating egg white during which the incorporation of air bubbles turns the translucent liquid white.

The mixer in use in this laboratory is similar to that of Fifield *et al.*, but is motor-driven at 57 rpm, while Fifield's is hand-operated, probably somewhat more slowly. It seems probable that 40 seconds of mixing in our machine is approximately equivalent to 60 seconds in theirs. Tests have shown that 40 seconds in the micro mixer is about equivalent to the 4-minute mix at 37 rpm used by Binnington and Geddes in the macro method.

Sheeting temperature: The temperature of the rolls, and thus presumably of the dough, during sheeting, appears to have a negligible effect on paste properties. An increase of 13°C in temperature decreased the absorption coefficient by only 0.05 unit.

Sheeting, number of times: In the micro test the sheeting and folding process corresponds to the kneading used in the macro test and in commercial plants. In a sense the sheeting and folding operation, or kneading for that matter, is an extension of the mixing period. There is this difference, however, that whereas mixing may well tend to incorporate air bubbles in the paste, and sheeting and folding may also do so, kneading should tend to remove the bubbles. Be that as it may, the effects of mixing and sheeting are similar; opacity increases with increases in either. The difference between pastes sheeted 5 and 20 times is about of the order that can be detected visually.

Binnington and Geddes studied the effects of kneading in the macro tests. The results obtained were somewhat erratic and thus difficult to interpret. In general they found that the effect of differences in kneading was considerably less than that of differences in water absorption.

In the micro test it is desirable to obtain a sheet of dough of uniform thickness. To attain this end the dough must be sheeted and folded a number of times. Moreover, the writers believe that the sheeting and folding serve as an additional and less rigorous mixing, during which greater uniformity is developed in the dough. Experiments have shown that the speed at which the rolls are turned and the consistency of the dough affect the thickness of the sheet and thus of the finished disk. Faster rolling and stiffer doughs produce thicker disks. It therefore seemed both advisable and convenient to drive the rolls mechanically (at 45 rpm) rather than by hand. Fifteen times through the rolls has been selected as adequate for the preparation of a sheet of uniform characteristics. The time element is standardized by sheeting at 6-second intervals.

In this connection it should also be noted that Fifield *et al.*, who sheet 20 times, claim that an interaction exists between varieties and sheeting. The difference between Mindum and Golden Ball is accentuated with increases in the number of times the dough is sheeted. This point has not yet been confirmed in this laboratory.

Pressure: Increasing the pressure to which the disks are subjected decreases their opacity. Under the conditions of the present experiment, doubling the pressure reduced the absorption coefficient by almost 50%. It thus seems safe to say that pressure is by far the most important of the processing factors studied.

This point is also stressed by Fifield *et al.* These authors recommend 8000 pounds per square inch for 30 seconds, but under these conditions the disks "were more translucent than the tubular macaroni made from the same sample." It was reported by LeClerc (1933) that pressures used in commercial practice range from 2500 to 5000 pounds per square inch. However, the actual pressure under which the dough is extruded is not easily determined since a large proportion of the pressure applied to the top of the dough in a cylindrical press is carried by the walls of the press rather than by the dough at the bottom of the press. Moreover, pressing conditions are not completely specified unless data on the time factor are given.

In selecting the conditions used in this laboratory the writers were governed by a number of considerations. In the first place, preliminary experiments had indicated that an interaction exists between processing conditions and different semolinas. Thus if semolinas were to be placed by the micro test in essentially the same order as in commercial practice, it appeared necessary to process under similar conditions. However, the two procedures differ so widely that an *a priori* selection of conditions in the micro test, which would be similar to commercial conditions, seemed impossible. Thus the best available

criterion appeared to be the similarity in appearance of the finished disks and macaroni. If high pressures are used the disks are far different in appearance from macaroni, being much more translucent and free from minute bubbles, and accordingly it seemed wise to work with a lower pressure range.

In practice the time the disks were to be held in the press was first established. A longer time than 30 seconds was selected, partly because this conforms more closely with commercial practice, and partly because it should serve to offset the elimination of the rest period used by Fifield *et al.* Seven minutes was finally selected because this time fitted well with the routine of preparing the disks.

Having established the press time, the pressure was then selected so as to give disks which had similar quality characteristics to tubular macaroni as judged by visual examination of the surfaces and of transverse sections, with respect to both texture and color. In this connection it should be noted that direct comparison of the colors of disks and macaroni with a comparator is essentially impossible because of the shadow effects in the tubular macaroni. Moreover the opacity of tubular macaroni cannot be measured by means of the photometer used for disks. It was thus necessary to depend mainly on visual comparisons. The pressure finally selected as a tentative standard for routine tests was 1000 pounds per square inch on the gauge of the Carver press (*i.e.*, on the ram), which is equivalent to about 700 pounds per square inch on the disks. Further experiments may show, of course, that some other combination of time and pressure will prove better or more convenient.

It will be apparent that in order to obtain reproducible results in the micro test it is essential that a standard pressure be used. This involves the use of a sensitive pressure gauge, the accuracy of which should be checked from time to time. The press itself should be kept in first-class condition. For instance, if leaks develop so that excessive periodic pumping is required to maintain the pressure, this has a packing effect on the disk which decreases the opacity slightly. The writers believe that some of their early difficulties in obtaining reproducible results can be traced to trouble with the press.

Press time: As would be expected, additional time in the press has the same effect on opacity as increasing pressure. However, doubling the time has far less effect than doubling the pressure. In practice it would appear satisfactory to select a press time convenient for making a series of disks under routine conditions and then select the pressure required to obtain disks of the desired quality.

Fifield *et al* note that the time in the press is important and should not be less than 30 seconds. They also say that a longer application of

pressure is required if the dough has been mixed slightly dry. It is apparent that these investigators, by using very high pressure, endeavored to develop maximum translucency in the paste. The curve for pressure and opacity levels off at high pressures so that the effects of small changes in pressure and press time are less important in the high range. The present writers believe that it is desirable to produce disks more closely similar to macaroni and that lower pressures must therefore be used in the micro test. Under such conditions both pressure and time in the press must be closely standardized if reproducible results are to be obtained.

Interaction Between Processing Factors

The statistics in Table III show a number of large interactions between various processing factors. The effect of a given change in one factor is not constant but depends upon the level of some other

TABLE III

ANALYSIS OF VARIANCE OF ABSORPTION COEFFICIENTS

Variance due to	Mean square	Variance due to	Mean square	Variance due to	Mean square
A Absorption	24.70‡	A × C	1.65‡	A × C × E	0.42‡
		A × D	0.12	A × C × F	0.23†
B Mixing temperature	0.67‡	A × E	2.12‡	A × D × E	0.07
		A × F	17.92‡	A × D × F	0.05
C Mixing time	88.43‡	A × G	0.71‡	A × E × F	1.92‡
		B × D	0.06	A × F × G	0.27†
D Sheeting temperature	0.07	B × F	0.57‡	B × D × F	0.07
		B × G	0.43‡	B × G × F	0.17†
E Sheeting, number of times	1.11‡	C × E	0.26†	C × F × G	0.09
		C × F	74.86‡	D × E × F	0.20†
F Pressure	221.63‡	C × G	0.21†	D × F × G	0.12
		D × E	0.19†		
G Time in press	6.68‡	D × F	0.23†		
		E × F	2.75‡		
		E × G	0.06		
		F × G	6.68‡	Remainder	0.0414

Note: Each portion of the variance has one degree of freedom except the remainder which has 93. † and ‡ denote that the 5% and 1% levels of significance are exceeded.

factor or factors. As pressure has the largest effect on paste properties it is not surprising that it is also involved in the major interactions. It is thus convenient to deal first with the interactions between pressure and other factors, discussing these in decreasing order of magnitude. There are only a few other interactions, not involving pressure, large enough to merit discussion.

In one or two instances interactions have been illustrated not only with data from the present experiment, but also by means of graphs based on other experiments in which more levels of one of the factors were studied.

Pressure × mixing time: Reference to the results of the analysis of variance (Table III) will show that the variance due to the interaction between pressure and mixing time (75) is exceeded only by the variance due to pressure (221) and to mixing time (88). Data illustrating the interaction are shown in Table IV. With increased mixing time the disks become more opaque (*i.e.*, the absorption coefficient is higher). This effect is enormous when the disks are subsequently pressed at 800 pounds per square inch, but is very small at a pressure of 1600 pounds. Alternately, it can be said that the effect of doubling the pressure is far smaller when the dough is mixed for 40 seconds than when it is mixed for 100.

TABLE IV

ABSORPTION COEFFICIENTS ILLUSTRATING THE INTERACTION BETWEEN PRESSURE AND CERTAIN OTHER FACTORS

		Pressure, lbs./sq. in.		
		800	1600	Difference
Mixing time (*seconds*)				
	40	3.91	2.80	1.11
	100	7.10	2.94	4.16
	Difference	−3.09	−0.14	
Water absorption (%)				
	28	6.32	2.94	3.38
	32	4.69	2.80	1.89
	Difference	1.63	0.14	
Press time (*minutes*)				
	3	5.90	2.93	2.97
	8	5.10	2.81	2.29
	Difference	0.80	0.12	
Number of sheetings				
	5	5.26	2.92	2.34
	20	5.74	2.82	2.92
	Difference	−0.48	0.10	

The interaction is illustrated in Figures 1 and 2, which represent experiments made with different semolinas. Figure 1 shows data for semolina E, processed at two different pressures and at a number of different mixing times, while Figure 2 shows data for semolina B processed at two mixing times and a number of different pressures. The second graph is the more illuminating. It shows that the curves for pressure tend to level off at about 1000 pounds per square inch and that the interaction between pressure and mixing time occurs largely below this level.

So far as can be determined by examination of disks, increasing the mixing time causes an increase in opacity largely as a result of the incorporation in the dough of many minute bubbles. With lower mixing times the bubbles are fewer and larger. The application of pressure subsequently tends to decrease the number of bubbles and enlarge

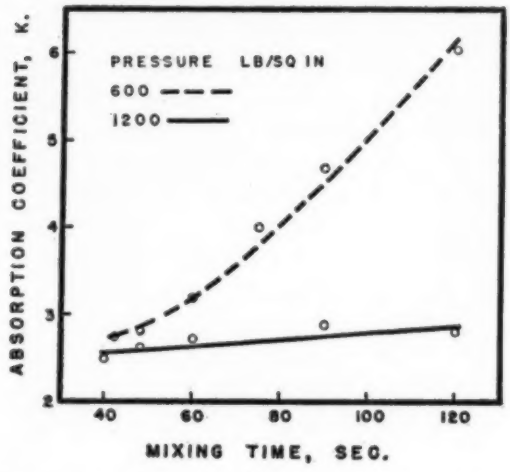

Fig. 1. Curves showing the effects, on the optical absorption coefficient of micro disks, of mixing for different times and pressing at 600 or 1200 pounds per square inch.

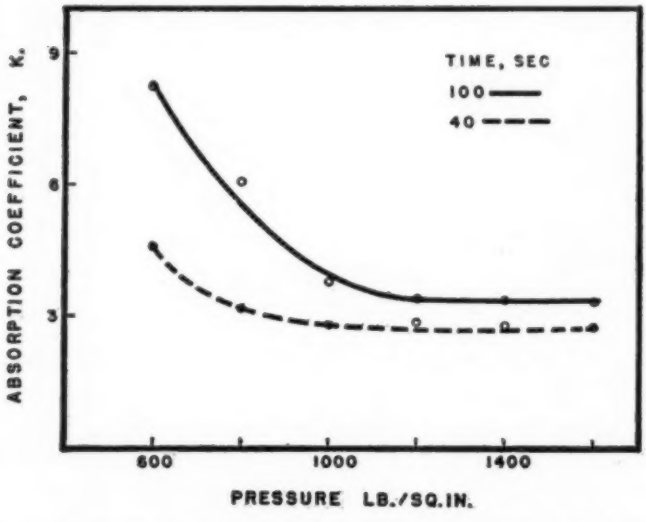

Fig. 2. Curves showing the effects, on the optical absorption coefficient of micro disks, of using different pressures after mixing for 40 or 100 seconds.

their size. Thus with paste mixed for 100 seconds, doubling the pressure reduces the opacity enormously, as there are many bubbles in the paste prior to pressing. But with doughs mixed for 40 seconds, doubling the pressure has a much smaller effect on opacity because there are fewer original bubbles. This appears to be the principal explanation of the interaction between mixing time and pressure.

Pressure × water absorption: Data illustrating the interaction between pressure and water absorption are also shown in Table IV. The effect of a 4% change in absorption is much less at 1600 pounds per square inch than at 800 pounds. The average difference between the disks at the 1600-pound level is of about the order which can just be detected by visual inspection. At the 800-pound level a wide difference in quality between disks having absorption of 28% and 32% is readily apparent.

The relation between absorption and pressure is better illustrated with data from another experiment in which semolina E was processed at two pressures and a wide range of absorptions. The data are shown in Figure 3. It is apparent that the effect of increasing absorption is much less at 1200 than at 800 pounds per square inch. Moreover, at

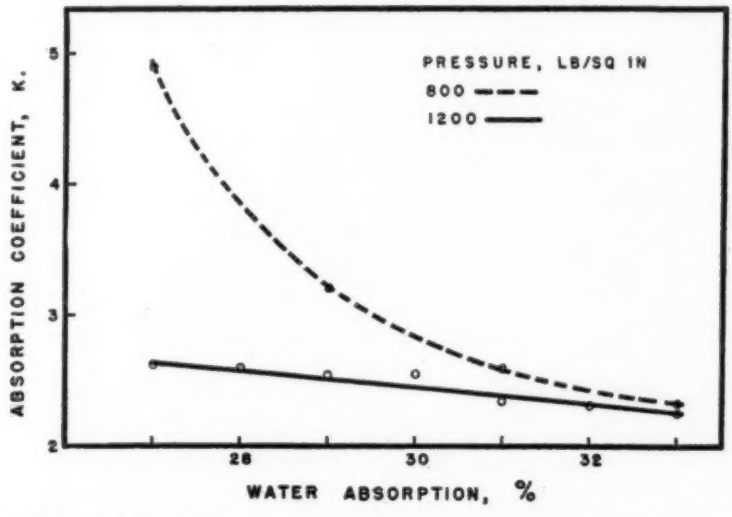

Fig. 3. Curves showing the effects, on the optical absorption coefficients of micro disks, of using different water absorptions and pressing at 800 or 1200 pounds per square inch.

the latter pressure level, a change of 1% in absorption has a far greater effect in the range below 30% than in the range above 30%.

Pressure and press time: Data illustrating this interaction are given in the third section of Table IV. As might be expected, at high pressure the length of time the disk is held in the press has a comparatively small effect on the quality of the paste, whereas at low pressures an appreciable change in quality can be obtained by varying the time during which pressure is applied. It will be noted that the interaction between pressure and press time is considerably smaller than the three interactions discussed previously.

Pressure and number of sheetings: The lower section of Table IV illustrates the interaction between pressure and the number of times the dough is folded and passed through the sheeting rolls. It will be observed that at 800 pounds of pressure an appreciable increase in opacity occurs between 5 and 20 sheetings. On the other hand, at 1600 pounds of pressure the opacity is slightly reduced by increased sheeting. This change in the effect of sheeting at different pressures is not readily explained. The writers are inclined to believe repeated folding and sheeting may tend to increase slightly the number of bubbles incorporated in the dough between the various layers of paste. At low pressure these layers may not be completely pressed together, so that an increase in opacity results from additional sheeting. On the other hand, at higher pressures this effect is overcome and the compacting of the dough within layers during sheeting offsets the possible inclusion of air bubbles between layers, thus decreasing the final opacity of the discs.

While no proof of this hypothesis can be offered, and the interaction is certainly small, further consideration of the points involved is merited. It seems doubtful whether the folding and sheeting technique of the micro test can be considered equivalent to the kneading used in the macro test and in commercial practice. It is certain at least that the latter process must tend to compact the dough and remove air bubbles, while it is by no means certain that this occurs in the sheeting procedure of the micro test. It seems possible that a complete change in technique may be advantageous at this stage of the micro processing method.

Absorption and number of sheetings: Data illustrating this interaction are given in Table V. The results are curious. At 28% absorption

TABLE V

ABSORPTION COEFFICIENTS ILLUSTRATING THE INTERACTION BETWEEN WATER ABSORPTION AND CERTAIN OTHER FACTORS

		Water absorption, %		
		28	32	Difference
Number of sheetings				
	5	4.40	3.78	0.62
	20	4.85	3.71	1.14
	Difference	−0.45	0.07	
Mixing time (*seconds*)				
	40	3.91	2.80	1.11
	100	5.34	4.69	0.65
	Difference	−1.43	−1.89	
Press time (*minutes*)				
	3	4.93	3.90	1.03
	8	4.32	3.59	0.73
	Difference	0.61	0.31	

an increase in the number of times the dough is folded and sheeted increases the opacity considerably. At 32% absorption an equal change in sheeting decreases the opacity very slightly.

This interaction does not appear to result from a differential amount of drying during sheeting. Thus it might be expected that the wetter dough would dry more during the sheeting operation. But if drying is equivalent to reducing the absorption then the opacity should tend to increase more (or rather, to decrease less) in the dough with the higher moisture content. This does not happen.

The writers are again of the opinion that the effect results from the creation of layers in the past during sheeting. Opacity will be increased to the extent to which these layers persist in the finished disk, *i.e.*, to the extent to which the layers are separated by increased numbers of air bubbles. It seems reasonable to suppose that there will be a far greater tendency for the layers to persist in drier doughs, so that an interaction between absorption and the number of sheeting operations may be expected.

Absorption and mixing time: Table V also contains data illustrating this interaction. Increasing the mixing time increases the opacity somewhat more in the dough with the higher absorption. Alternately, it can also be said that the effects of a 4% increase in absorption are considerably greater with 40 seconds of mixing than with 100 seconds. Although it may appear to be laboring the point, the writers believe that this interaction can again be explained in part by the number and size of the air bubbles incorporated in the paste. However, other and less easily observed effects of absorption and mixing on the characteristics of the doughs must also be expected to contribute to the interaction.

Absorption and press time: The data in the lower section of Table V show that the interaction between absorption and press time is similar to that between absorption and pressure. With increased press time, as with increased pressure, the effect of a 4% increase in absorption decreases. There appears to be little doubt that the effects of increasing the time during which pressure is applied are essentially the same as the effects of increasing the pressure.

Other first-order interactions: Most of the remaining interactions (see Table III) are too small to merit separate discussion. Two attained the 1% level of significance, four attained the 5% level, and the remaining ones were not significant.

The two interactions, not yet discussed, which attained the 1% level involve mixing temperatures and pressure. It appears that a differential effect of pressure and press time exists on doughs mixed at high and low temperatures. The interactions, however, are comparatively small.

Of the four remaining interactions which were significant, two involve mixing time, which interacts with press time and the number of sheetings, and two involve sheeting temperature, which interacts with pressure and also with the number of sheetings. None of these interactions is of sufficient practical importance to merit discussion.

Second-order interactions: Reference to the statistics in Table III will show that only one of the second-order interactions is large, namely, that between pressure, absorption, and number of sheetings. Pressure is the factor causing the greatest change in paste properties and the interaction between absorption and sheeting is the largest first-order interaction not involving pressure. Accordingly, it is not surprising that the second-order interaction under consideration should attain considerable magnitude. It will also be noted that the next largest second-order interaction merely involves the substitution of mixing time for pressure (*i.e.*, mixing time × absorption × sheeting).

It is perhaps surprising that none of the second-order interactions involving both pressure and mixing time is large, in view of the size of the first-order interaction involving these two factors. Actually, only one of these second-order interactions attains the 5% level of significance, namely, that which also involves absorption.

A knowledge of the second-order interactions is of comparatively little practical use in establishing a precise micro test or in designing experiments bearing on the utility of the test for studying different semolinas. Accordingly, presentation of data illustrating the second-order interactions, and discussion of them, does not seem warranted.

Discussion

The utility of the data published in this paper is obviously limited by the fact that they deal only with opacity measurements rather than with the more commonly measured color characteristics of the paste. However, it has been shown in the previous paper that, with disks made from one semolina, color is closely related to opacity; *i.e.*, the yellowness of the paste is inversely related to the optical absorption coefficient. Thus since each experiment in the present paper was made with one semolina, the data on opacity can be interpreted in terms of color. It will also be apparent that measurements of opacity are far more useful than color determinations in a study of the present type, since the former measurement is a great deal more precise and rapid. Thus many of the effects which can be readily demonstrated with opacity measurements, either as statistically significant differences or as smooth curves, could not be demonstrated by the less precise determinations of color. The errors of the final determination of color would have been so great as to mask real though small differences, and curves would

have been unconvincing because of their irregularity. There is, moreover, the added difficulty that the time consumed in making color measurement would have been so great that it would have been necessary to reduce the scale of the investigation. When large differences in paste properties were demonstrated by opacity measurements, these could also be seen by inspection as differences in the color of the paste.

In order to obtain the highest degree of reproducibility in the micro test, it appears necessary to provide close control of conditions at each stage of processing. The most important stages are as follows: pressing, during which pressure and time in press must be closely standardized; mixing, during which the speed, time, and temperature must be maintained at constant levels; and the amount of water added to the dough, which must be standardized, either at constant absorption or possibly at constant consistency. The number of sheeting operations and the drying temperature have less effect on paste properties but also merit control.

In general the authors would also recommend careful standardization of all other processing factors whenever this is feasible, particularly if strictly comparable results are required at widely different times (*e.g.*, for crops of different years). This recommendation is based on two premises: first, that the present investigation is not all-inclusive, and second, that no test can be too precise provided precision is not obtained at the expense of speed or convenience, or by the investment in equipment of more capital than is warranted to attain a practical level of reproducibility.

It is pertinent to note that the sampling error is probably the limiting error in the micro test when the standardization recommended in the last paragraph but one is adopted. The sampling error for semolina, though appreciably less than that for whole wheat, is greater than that for flour. However, semolina can be conveniently handled with a Boerner sampler, and this practice is followed in this laboratory. Nevertheless, so far as can be judged from the results of careful experiments, the sampling error is still a limiting factor in obtaining accurate and reproducible results. In certain applications of the micro test, notably in testing new hybrid wheats at an early stage, the sampling error will be that of wheat. This will occur when the plant breeder can supply only 100 g of wheat for testing, and all this must be milled to produce enough semolina for one mix. In this event the degree to which a single sample can represent the average performance of a variety will certainly limit the interpretation of results.

To return to the errors of the test itself, it should be pointed out that these can be divided into intra-batch and inter-batch (or inter-day) errors. The latter are greater than the former. Accordingly, statisti-

cal design of the investigation for the control of the inter-day error will frequently be required. Thus, if samples representing a number of varieties grown at several stations are to be tested with the main object of comparing varieties, then all samples from one station should be tested in one batch, and so on, so that the inter-batch error is removed from the comparison of varieties and placed with the less important comparison of stations. The micro test provides ample scope for such designs since single doughs, and duplicate disks, can readily be made from 16 different semolinas in a working day of $6\frac{1}{2}$ hours, together with measurements of the opacity of such disks from a previous batch as may be ready for measurement on that day. This rate presupposes a team of two, which is convenient for large-scale investigations with the micro method.

A study of the interactions between various processing factors suggests that the precision of the test will be affected by the levels at which certain of the factors are standardized. Thus at high pressures the effects of changes in mixing time and absorption are minimized; at low mixing times the effects of pressure changes are smaller though the effect of mixing is greater; and at high absorption the effects of pressure changes are again lower though the effects of mixing are greater. In general it appears that the most precise results can certainly be obtained at high pressures, and from this point of view it also appears best to work with a low mixing time and a high absorption.

However, the selection of levels for various processing factors must be conditioned by considerations other than the reproducibility of the results. A small preliminary study of the effect of processing conditions on different semolinas has shown that these can be placed in different order of rank with respect to opacity and color by varying the processing method. Under these conditions it follows that, since it is desirable that the micro test place semolinas in the same order as they would be placed in commercial practice, the micro test should simulate the conditions used in large plants and produce pastes of the same general properties.

The selection of suitable conditions for the micro test is no simple matter. It seems impossible to base the selection on *a priori* considerations owing to the wide differences in the techniques employed in the two procedures. For instance, in the micro test a short mixing time can be used because of the small amount of dough handled. On the other hand it may well be that longer and less vigorous mixing would be better. In the micro test the disks are pressed in confined space, whereas in commercial practice the dough is pressed through a die. It is extremely difficult to equate these two conditions. Again, in commercial practice the dough is kneaded for some time, but in the

micro test, as presently developed, kneading is replaced by repeated folding and sheeting, an entirely different process.

In the investigations so far reported, the writers had no need to adopt standardized levels for the various processing factors since it was the effect of changing these which was under investigation. Nevertheless, some tentative selection of levels has been made and is indeed implied merely by the ranges studied for each factor. The criterion used in the selection was that the pastes obtained should have the general appearance of commercially made macaroni when inspected visually either by examination of the surfaces of the disks and macaroni, or by comparison of transverse fractions.

The writers are not prepared to defend the present tentative selection of conditions vigorously. Indeed it may shortly be demonstrated that these should be changed. It is apparent that a great deal more investigation of a number of semolinas must be made before it can be shown conclusively that the micro test now used in the laboratory is a useful tool for assessing the quality characteristics of different wheats and semolinas. Though it is difficult to spare men and time for research in this field during the war, it is hoped that some further progress can be made in the near future.

Summary

In the micro test for alimentary pastes, doughs are mixed and sheeted and disks are then cut from the sheet, pressed and dried. A study has been made of the effects of processing conditions on paste properties, as measured by the optical coefficient of opacity, with the principal object of determining the degree of standardization that must be achieved at each stage of processing in order to obtain highly reproducible results.

The investigations show that the most important stages are: pressing, during which pressure and time in press must be closely standardized; mixing, during which speed, time, and temperature must be maintained at constant levels; and the amount of water added to the dough which must be standardized either at constant absorption or possibly at constant dough consistency. The number of sheeting operations and the drying temperature have less effect on paste properties but also merit control. Temperature of sheeting rolls and press have little effect. It is recommended that no rest period be used prior to pressing. Time of drying should be standardized, mainly because of changes in paste properties with time, which appear to be associated with aging rather than with the final moisture content of the disks. Considerable variation in the relative humidity of the air during drying does not appear to be important, presumably because the disks are

encased between sheets of bond and filter paper during this process, and because the final moisture content of the disks is not critical.

The elucidation of the effects of changing the processing conditions is complicated by the existence of interactions between various pairs of factors. It was necessary, therefore, to use factorial designs throughout the investigations and this made possible the study of the major interactions. The most important appear to be those between pressure and mixing time, pressure and absorption, and pressure and time in press. The existence of these interactions makes it impossible to state the exact effect on paste properties of a change at any one stage of processing unless the remaining processing conditions are also specified.

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MEASUREMENT AND SIGNIFICANCE OF GLUTEN QUALITY

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Gluten quality is a term broadly used in referring to variations in the bread-making properties of flours of the same gluten quantity. The apparent quality of gluten may be altered by the treatment and handling of dough in the baking process. The reasons for gluten quality variations and alteration by different treatment such as oxidation and reduction, fermentation, proteolytic enzymes, etc., have been matters of much interest to cereal chemists. Many believe the changes brought about by oxidizing and reducing treatments are due to hydration or alteration of the gluten itself, while others consider that they may be largely a result of changes in nongluten constituents, such as water solubles and lipids, which are closely associated with the gluten.

Investigations of physical properties of gluten have been under way for some time in our laboratory, and a method of testing these proper-

ties of gluten has been described by Baker, Mize, and Parker (1942). At one time the method appeared suitable for examining the physical characteristics of gluten and for testing the effects of different modifying treatments. It became apparent after further use that the method, though satisfactory for earlier work, was not accurate enough for more exacting requirements. A new apparatus was devised by modifying the machine designed by Baker for testing the physical properties of bread, and described by Platt and Powers (1940).

Apparatus and Procedure

The Baker instrument was modified to carry a probe having at the end a small ball, 1 mm in diameter, which was made to penetrate a gluten ball by force applied from a motor. A special holder for the gluten was developed, so that testing conditions on different samples would be as nearly constant as possible. The photograph (Fig. 1)

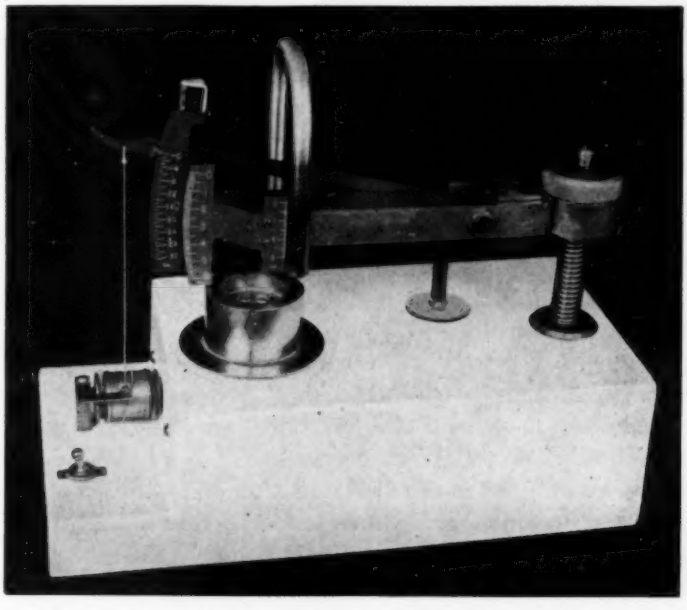

Fig. 1. Modified instrument for the measurement of force required to puncture a gluten ball.

shows the apparatus, with the gluten under strain in position in the testing chamber and the probe resting on one of the test points at which the gluten is to be penetrated. When the motor is started, the cord is drawn down over the drum at a constant rate; this motion is applied through a spring to the arm carrying the probe, which is thus forced downward into the gluten. When the gluten is properly pre-

pared for testing in this manner, the probe forces the test surface inward without penetrating, until the surface can no longer resist the penetrating force and suddenly yields, permitting the probe to enter with an abrupt increase in the rate of the downward motion of the probe. One can read on the scale the force, in grams, at which this penetration of the surface occurs. The readings thus taken indicate the force in grams required to cause a 1-mm ball to penetrate the surface of the prepared gluten. Penetration occurs only when the surface has reached its elastic limit and rupture of the surface takes place.

Glutens were washed from doughs under standardized conditions. A half-hour resting period was allowed after the dough was mixed in order that gluten might be obtained from flours which washed with difficulty. In other instances fermented doughs were washed after a prescribed period of fermentation. The gluten from 200 g of flour was washed in the presence of carbon dioxide in 2 l of 3½% salt solution at 75°F and allowed to rest for 30 minutes. The gluten was then formed into a ball weighing 23 g and repeatedly, gently grasped in the hand and extruded between the thumb and forefinger of the closed fist in such a manner that the ball of gluten thus formed was stretched, though not enough to break the surface.

The extrusion was carried on very gently, the finger reopened, permitting the gluten to return, and the gluten again extruded many times to gradually force it to assume a smooth, glistening, unbroken spherical surface under strain. The ball was then closed at the base by squeezing with the finger and quickly placed in the test dish where a heavy ring was placed on top of the gluten to hold it in the strained position beneath the surface of a 3½% salt solution.

A plate with holes for six test positions was placed in position to hold the gluten and the ring in place. Six penetrations of the prepared ball were made and the force in grams required to penetrate noted. These readings were averaged. The sample was permitted to rest one hour, and the test repeated after the sample was again prepared in the above manner. Three to five such tests were made, giving a total of 18 to 30 readings on a single gluten. All exceptionally low readings were discarded, the result reported being the average of the higher readings. Low readings were discarded because they indicated flaws or weak points in the gluten which happened to exist at the point of test.

In order to purify the gluten, 70 g of the sample after testing was placed in a Waring Blendor with 400 g of ice and 3 g of salt. The entire apparatus was then placed in a vacuum chamber, and the Waring Blendor was allowed to run at full speed for five minutes to disperse the gluten into a fine state of subdivision, producing a suspension of

milky appearance. Then the vacuum was released with carbon dioxide, the gluten suspension poured into cups and centrifuged, where it reformed to a mass that could be collected and handled as ordinary gluten. In this process the water solubles and the fine starch particles which are ordinarily impossible to wash from gluten are largely removed. The gluten was then tested in the machine, again dispersed and this process repeated until the readings obtained reached a maximum, indicating that the purification of the gluten by dispersion had been approximately completed. Glutens are purified under these conditions after three dispersions.

The total amount of salt solution used in the entire process of washing and dispersing the gluten from 150 g of flour was 2,700 ml. This volume is kept low to minimize the amount of gliadin dissolved.

Measurements, Preliminary and General

The results of the successive dispersions can be plotted in grams required to penetrate the gluten. Figure 2 shows three series of results

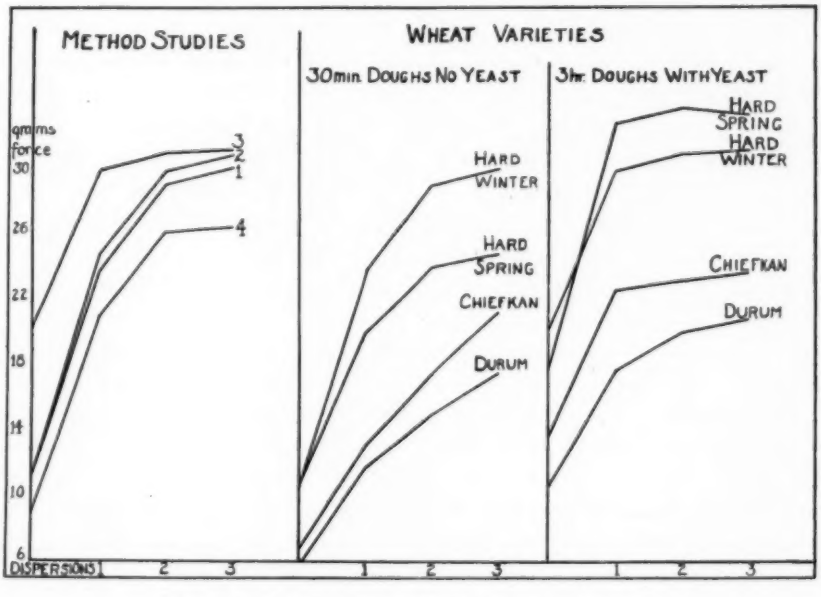

Fig. 2. Relation of gluten purification to puncturing force.

obtained by this method. The first series was designed to show the application of the method. Curve 1 gives the results obtained by dispersing a gluten washed under the above specified conditions. On the ordinate is plotted the first point for each curve and shows the force

required to puncture the undispersed gluten as it was obtained after gluten washing. The gluten was tested and the puncturing force was measured after each of the three successive purifying dispersions. This puncturing force increased until substantially constant value was obtained after the third purification. Dispersions beyond the third often gave results lower in value than the maximum. This decrease in strength on further purification may have been due to the occasional slight leakage of oxygen into our vacuum apparatus.

Curve 2 in the first series shows the effect on the gluten strength of extracting flour fats from the same flour by the method described by Baker, Mize, and Parker (1942). Improvement obtained was no greater than one would expect from the removal of inert material equivalent to this amount of fat, resulting in a greater concentration of gluten in the sample. This study thereby confirmed our opinion that the extractable fat in sound flour exerts very little effect upon gluten quality.

Curves 1 and 2 were obtained by mixing a dough without yeast and permitting it to stand 30 minutes before washing. The third curve shows the effect of three-hour fermentation before the gluten was washed. The fermentation seemingly permitted greater purification of the gluten during gluten washing. The strength of the fermented hand-washed gluten was nearly double that from an unfermented 30-minute dough. Upon dispersion, this fermented gluten was more quickly purified to a maximum strength which was somewhat greater than is obtainable with unfermented gluten. This was probably due to the removal of glutathione from the system by the yeast during the fermentation, as described by Hulett (1940), thereby eliminating the effect of glutathione upon the proteins as reported by Sullivan, Howe, Schmalz, and Astleford (1940). There was also an improvement of the gluten due to the more thorough removal of the small starch granules from the gluten during the washing of a fermented dough.

The fourth curve illustrates the effect of autolysis without yeast. In this case the dough stood three hours in a manner similar to that shown in Curve 3, but in the absence of yeast. Here the autolytic effect was to lower the strength of the gluten at all points of purification. This was possibly due to proteolytic action in the absence of yeast.

The second series of curves shows the results with washed and purified glutens from a series of widely divergent flours. These glutens were prepared from 30-minute doughs containing no yeast. The freshly washed glutens were all fairly weak. As the purification proceeded, all of the glutens improved in strength until a maximum was reached at the third dispersion. These studies clearly suggest that the differences among the baking properties of these wheats (see Table I)

are primarily due to the inherent qualities of their glutens. The magnitudes of the "strengths" of these glutens do not appear to be in the exact order of baking quality inasmuch as the bread prepared from hard spring flour was superior to any of the others in this series.

Results with glutens washed from doughs that had been fermented are shown in the third series. The purified gluten from the hard spring wheat resisted puncture more than any of the others. While the readings obtained for the hand washed glutens before dispersion in either series do not indicate the baking order of these flours, those of the purified fermented glutens do seem well correlated with baking values. This evidence is interpreted to mean that the character of the gluten is the variable that controls flour quality and suggests that water solubles play a minor part.

Effect of Additions of Various Ingredients

Table I gives an analysis of the water solubles in the supernatant liquor obtained by centrifuging a batter of two parts water and one part flour. In this table are given the quantity of total solids, soluble proteins, soluble pentosans, the maximum force required to puncture the purified gluten from fermented dough, and the loaf volume and texture of the bread obtained from these flours.

TABLE I

ANALYSIS OF WATER SOLUBLES IN BATTERS

Flour	Solubles in supernatant from a 2 to 1 batter			Gluten	Bread	
	Total solids	Soluble protein	Soluble pentosan	Maximum puncture force	Loaf volume	Texture
	g per 10 ml			*g*	*cc*	
Hard spring	0.3844	0.0780	0.0554	33.4	2840	102
Hard winter	0.3638	0.0762	0.0596	31.0	2570	99
Chiefkan 1939	0.2874	0.0668	0.0384	23.7	2280	90
Durum	0.6204	0.0808	0.0580	20.8	1900	80

The puncture-force values are in direct relationship to the baking results. The analysis of the water solubles shows little correlation with baking value, but this does not imply that the solubles are without effect.

Bread was baked with a "dough-up" liquid consisting of the concentrated flour extract from the sound flours above described, whereby the concentration of flour solubles in the dough was increased by about 25%. With proper treatment, such as adjustment of oxidation or fermentation, the use of flour extract improved the quality of bread

obtained from a given flour. It is thus evident that some flour solubles may be desirable in bread baking and it seems probable that the more of these desirable solubles a dough contains, the better the bread that may be produced. This is presumably due to the effect of the increased concentration of solubles upon the viscosity of the dough. Heating the extract to the clouding point of the leucosin further improves the baking value, indicating that leucosin may not be entirely desirable.

Figures 3 and 4 show the force required to puncture glutens obtained from the use of sprouted wheat and from additions of other ingredients to dough. The first series of curves in Figure 3 shows results with glutens, respectively, from a sound flour and from flours milled from wheat containing 10% and 27% of sprouted grain. These flours were all obtained from hard spring wheat of the same area, and were of substantially similar character. The presence of 10% of sprouted grain did not produce much effect upon the ultimate strength of the gluten, suggesting that sprouting in its early stages does not produce much proteolytic enzyme. Because of the presence of alpha-amylase and deteriorated pentosan, the presence of sprouted grain does have an effect upon bread baking, as shown by Baker, Parker, and Mize (1943). With the flour made from wheat containing the larger percentage of sprouted grain, the recovered gluten showed considerable effect of proteolytic activity, resulting in quality deterioration, as shown in the lower curve.

The second series of curves in Figure 3 shows the effect of added malt. In this case 0.5% malt flour was added, which is in excess of commercial usage. In malted flour, where the sprouting process has been carried much further than is found in ordinary sprouted grain, a considerable amount of proteolytic enzyme developed and deterioration of the gluten resulted.

The third series of curves in Figure 3 shows the effect of adding bromate to the dough before fermentation. The upper curve (No. 1) is the control curve for the one containing no bromate. Curves 2, 3, and 4 are for those containing 10, 20, and 80 ppm of bromate respectively. The larger amounts of bromate have produced deterioration in gluten quality.

Curve 5 shows the effect of using milk powder when 80 ppm of bromate is used in baking. The washed gluten strength is similar to that of the gluten from a dough containing 80 ppm of bromate. However, this gluten retained substantially all of its strength after having been thoroughly purified by three dispersions. This may explain the findings of Ofelt and Larmour (1940), who show that milk prevents the damage produced by excessive amounts of bromate.

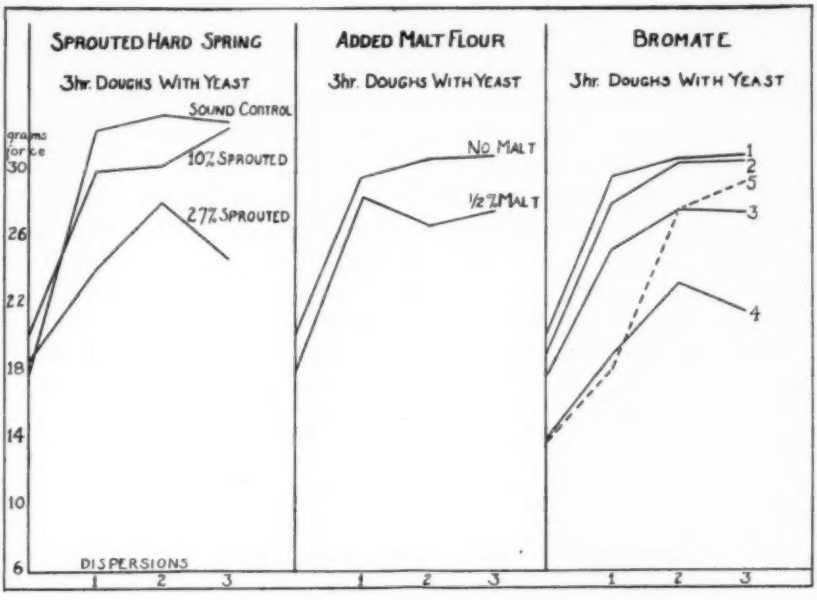

Fig. 3. Relation of gluten purification to puncturing force—glutens obtained from a sound flour and from flours containing 10% and 27% of sprouted grain.

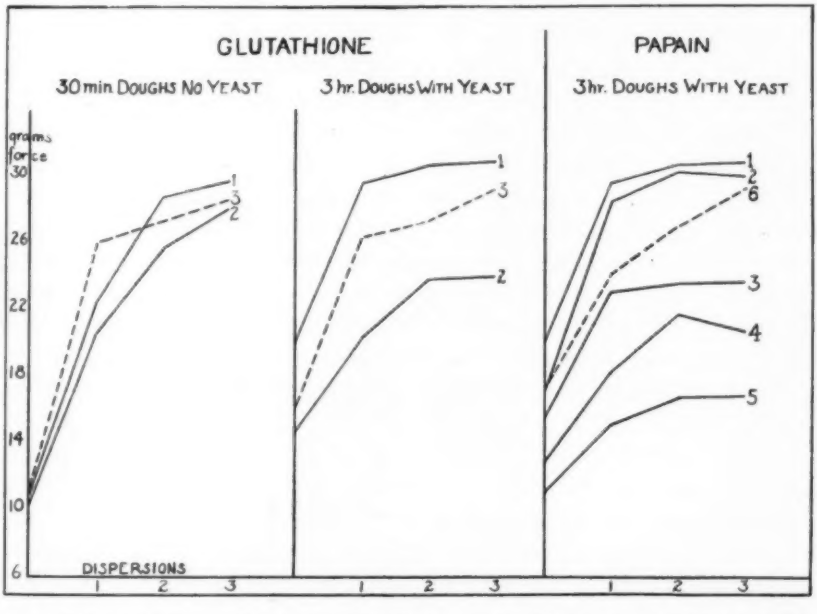

Fig. 4. Relation of gluten purification to penetrating force—glutathione and papain added to doughs.

Figure 4 shows the results of adding either glutathione or papain to doughs; also, the action of subsequent oxidation upon such doughs. The first series gives the results obtained with glutathione in a 30-minute dough without yeast. Curve 1 is from untreated flour. Curve 2 shows that glutathione produces a lowering of gluten strength. Curve 3 gives the effect of oxidizing the glutathione dough by washing it in water containing a small amount of sodium chlorite. The use of the oxidizing agent has returned the gluten in part to its original strength, though complete recovery was not obtained.

The next series of curves shows the results obtained by fermenting these same doughs three hours with yeast. The lower curve (No. 2) shows that the added glutathione had a very marked effect in the three hours, even in the presence of yeast. This was much greater than occurred in the 30-minute dough shown on the previous curve, indicating that the yeast was unable to consume all the glutathione. The excess glutathione either activated the proteolysis in the dough or else continued to alter the gluten molecules so that much weaker gluten was obtained. Curve 3 shows the effect of washing the gluten from this dough in water containing sodium chlorite. Apparently the oxidizing agent reversed the effect produced by the glutathione and returned the gluten again toward its original properties, though there was still some loss in strength.

The next series of curves shows results obtained from the use of papain in fermented doughs, the top curve representing no papain and the remaining curves reflecting increasing increments of papain. The smallest amount of papain used (5 ppm, Curve 2) had little effect. The larger amounts of papain (10, 20, and 40 ppm, Curves 3, 4 and 5) showed increasing damage to the glutens.

Curve 3, representing 10 ppm of papain, shows the first marked deterioration of gluten quality, similar to that obtained by the 50 ppm of glutathione. When this dough was washed in water containing sodium chlorite (Curve 6) the injury produced by papain was reversed and the gluten returned nearly to its original strength. This gives an indication of the manner in which glutathione and papain act on gluten, and also of the manner in which oxidizing agents affect those agents. Both the glutathione and the papain apparently begin the disintegration of gluten in a similar manner and cause loss of "strength."

The results presented here illustrate the possibilities of this method of testing gluten. Many other variables could have been applied to doughs and tested by the same method. We expect in the near future to make a similar study on the use of flour oxidants as contrasted to the use of dough oxidants here reported.

Discussion

Gluten quality can now be defined and denoted in terms of its resistance to rupture as measured by the puncture test on purified gluten. The full strength of gluten is not evident until it has been subjected to fermentation, which eliminates glutathione,[1] releases the small starch from the gluten, and assembles the gluten prior to washing. Gluten, when developed by yeast in the dough, seems to exhibit its maximum strength. The apparent strength of gluten may be affected by the solubles in the flour. In general, these solubles do not vary greatly in amount among different flours. The baking character of a flour can be varied by introducing solubles extracted from flour. The addition of ingredients to doughs may alter dough properties or may promote the speed at which various undesirable characteristics of a dough are eliminated during fermentation. Thus oxidation of the glutathione reduces this task for the yeast. No ingredients or treatments which may be added to the dough produce an increase in the potential strength of such gluten. They either lower the gluten strength or have no effect. The improving action of certain ingredients which are used in dough must be due to effects other than a direct action on "gluten quality" as here defined.

Gluten quality is a wheat variety characteristic; the better wheats, as adjudged from a bread making standpoint, have stronger gluten. Glutens from some wheats, such as durum, are so weak, as here measured, that they are incapable of making good bread.

The potential bread-making capacity of a given flour should be determined by the ability of its gluten to resist rupture. The capacity of such glutens to make improved bread can be shown by use of the "no dough time" method (Baker and Mize, 1941). In standard bread-making procedure, however, the effect of increased gluten strength may in many cases be undesirable, causing such things as bucky doughs.

The action and effect of oxidizing agents on dough can be further summarized. Combining the evidence submitted in this paper with the observations found in the work on pentosans by Baker, Parker, and Mize (1943), the first effect of an oxidizing agent in dough is the elimination of the "glutathione" type of reducing substances present, so that the gluten strength is improved. At the same time, the oxidation of the glutathione alters its usual effect upon soluble pentosans in the dough and they become more viscous, thus reducing the rate at which the gas bubbles in the dough may coalesce. The dough lubri-

[1] Where the word "glutathione" appears in this discussion to describe a natural flour constituent, we use the term to mean glutathionelike compounds characterized by the -SH group. We have not determined their identity.

cant becomes thicker because of the oxidation of the glutathione, and the flow of the dough substance is thereby decreased. After the glutathione is partially or wholly oxidized, the action of the oxidizing agent may continue upon the soluble pentosans themselves, further increasing their viscosity, and finally changing them into a gel form. The resultant thickening of dough should produce a rigidity that will resist deformation of cell structure. Hence, it may be desirable that the gelling reactions do not occur until after the dough has passed through all quick mechanical deformations during the baking procedure. Once it is in the pan, the rigidity will cause no further harm. This has been clearly shown in the work by Baker and Mize (1941) on the making of "no dough time" doughs.

When the glutathione has been oxidized, the action of the oxidizing agent may continue further and affect the proteins. Oxidizing agents act upon protein to lower its mechanical strength, causing gluten to be more easily punctured or broken. Excessive oxidation can produce a very material lowering of gluten strength. The addition of milk to doughs seems to prevent this action of the oxidizing agent upon the gluten. It also was shown in the experimental data that oxidizing agents can prevent papain from lowering the puncture strength of gluten. These observations support the assumption that oxidizing agents may cause decreased proteolysis when present in doughs, either by acting on glutathione or by directly attacking proteolytic enzymes.

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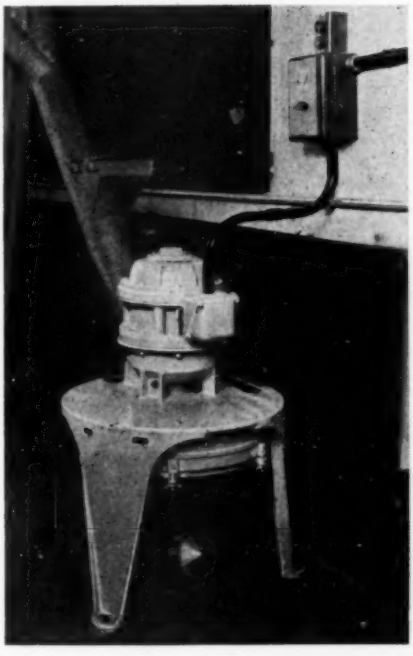

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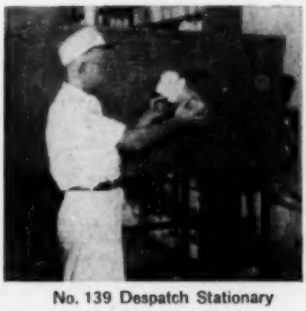

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